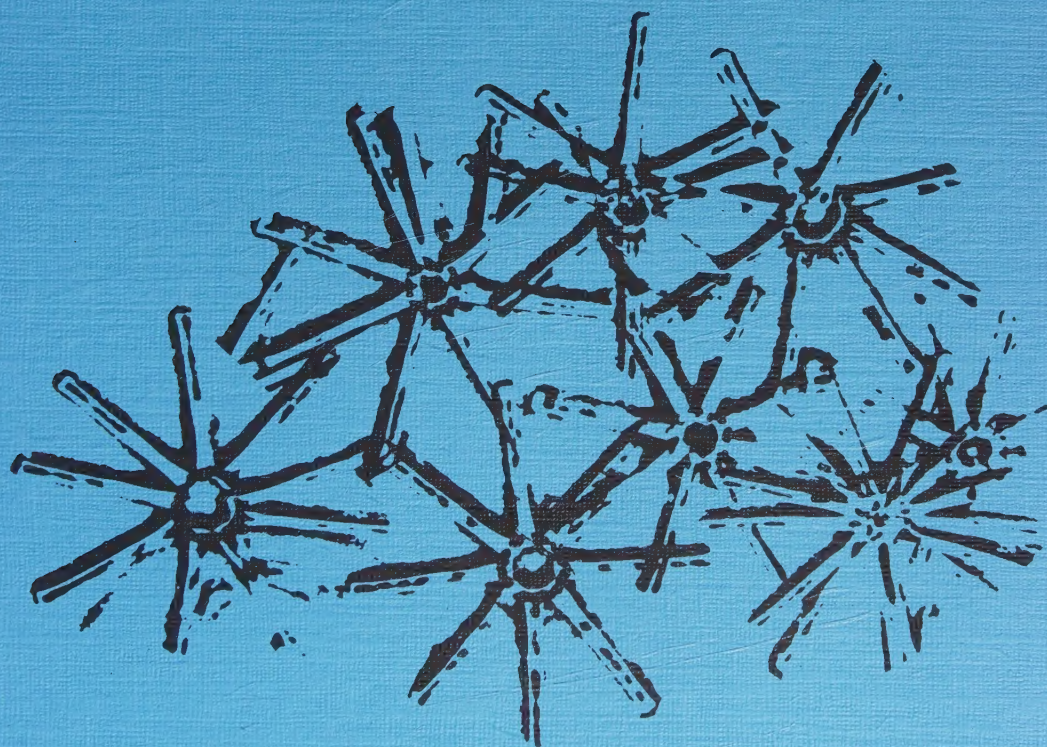




LIMNOLOGISKA INSTITUTIONEN
LUNDS UNIVERSITET

PHYTOPLANKTON PRODUCTION AND
TRANSPORT OF PHOTOSYNTHETIC
CARBON TO BACTERIA

Michael F. Coveney



INSTITUTE OF LIMNOLOGY
University of Lund
Box 3060
S-220 03 LUND Sweden

LUND
MAY 1980

PHYTOPLANKTON PRODUCTION AND TRANSPORT OF
PHOTOSYNTHETIC CARBON TO BACTERIA

av

Michael F. Coveney, Vrml

Akademisk avhandling som för avläggande av
filosofie doktorsexamen vid Matematisk-natur-
vetenskapliga fakulteten vid Universitetet i
Lund kommer att offentligen försvaras å Limno-
logiska institutionen, Fabriksgatan 2, Lund,
torsdagen den 29 maj 1980 kl. 10:15. Disputa-
tionen sker på engelska.



Dokumentutgivare
Limnologiska institutionen
Handläggare Lunds universitet
Michael F. Coveney
Författare
Michael F. Coveney

Dokumentnamn
DISSERTATION
Utgivningsdatum
May 1980

Dokumentbeteckning
Ärendebeteckning
LUNBDS/(NB LI-
1007)/1-12/(1980)

Statens naturvetenskapliga
forskningsråd
Box 23136, 104 35 STOCKHOLM
Statens naturvårdsverk
Fack, 171 20 SOLNA

Dokumenttitel och undertitel

Phytoplankton production and transport of photosynthetic carbon
to bacteria

Referat (sammandrag)

The dissertation is a summary of five published and unpublished papers. Phytoplankton 14-C productivity was measured *in situ* in eutrophic, hard-water Lake Bysjön, south Sweden. Changes in photosynthetic capacity and phytoplankton growth rate constants were related to temperature and nutrients. Separation of algae and bacteria by size fractionation was used in 14-C productivity determinations to estimate bacterial uptake of algal excretion products. This varied from 36 to 73% of 14-C released during 4 h incubation and changed little with depth in each experiment. Gross excretion *in situ* was 1.7 to 6.8% of areal 14-C assimilation on 7 occasions. Laboratory time-series incubations indicated steady state for the extracellular product pool in only 1 of 3 experiments.

Referat skrivet av
förf

Förslag till ytterligare nyckelord
dissolved organic matter, heterotrophic uptake, chlorophyll a

Klassifikationssystem och -klass(er)

Indextermer (ange källa)

Omfång
12

Övriga bibliografiska uppgifter

Språk
engelska

Sekretessuppgifter

ISSN
0348-0798

ISBN

Dokumentet kan erhållas från
Biblioteket
Limnologiska inst
Box 3060, 220 03 Lund

Mottagarens uppgifter

Pris

Blankett LU 11:25 1976-07

Digitized by the Internet Archive
in 2026

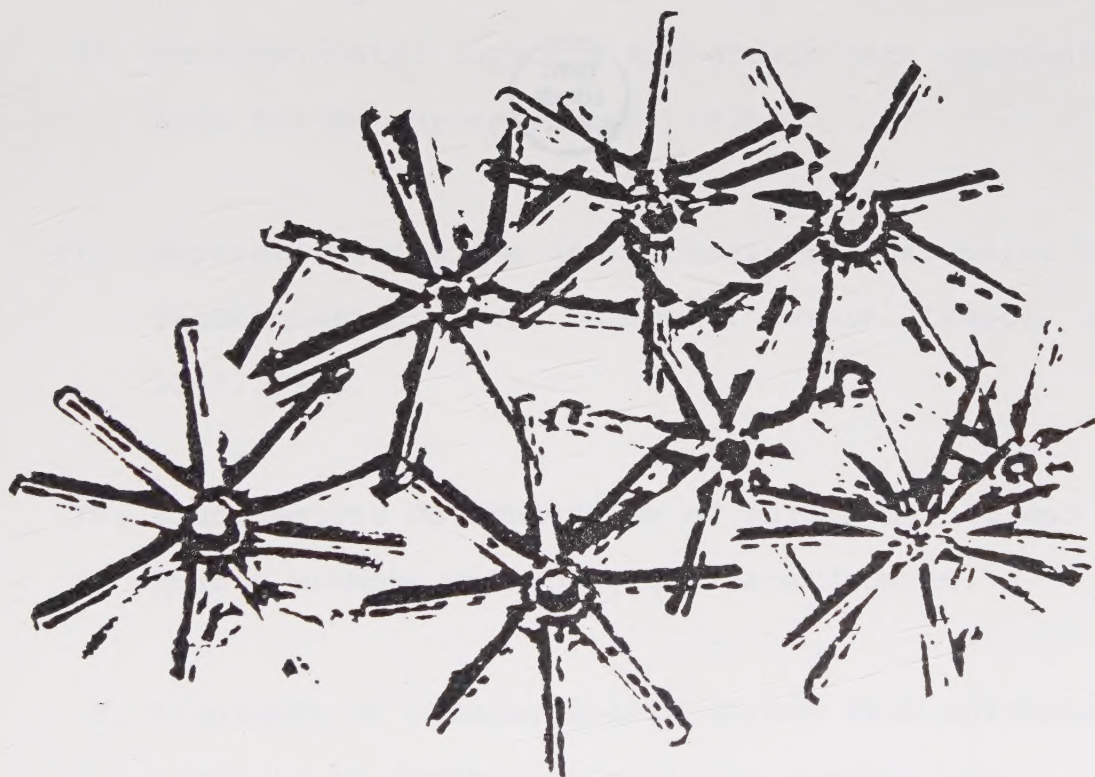
https://archive.org/details/IA41553422_0019



LIMNOLOGISKA INSTITUTIONEN
LUNDS UNIVERSITET

PHYTOPLANKTON PRODUCTION AND
TRANSPORT OF PHOTOSYNTHETIC
CARBON TO BACTERIA

Michael F. Coveney



INSTITUTE OF LIMNOLOGY

University of Lund

Box 3060

S-220 03 LUND Sweden

LUND 1980

CODEN LUNBDS/(NBLI-1007)/1-12 /(1980)

ISSN 0348-0798

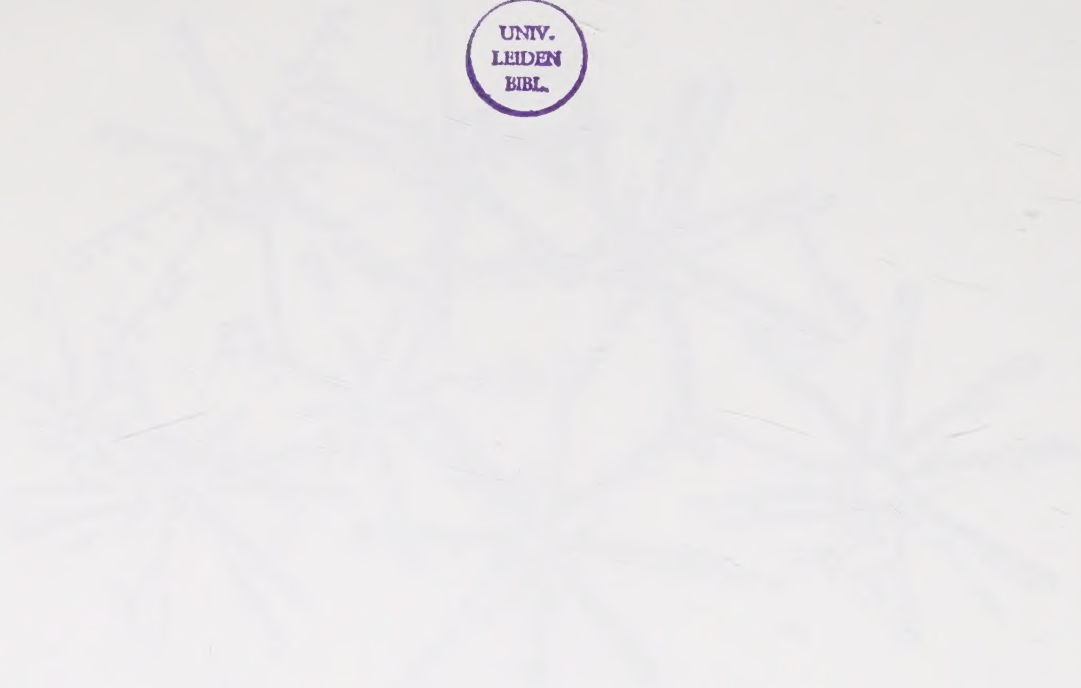


PHYTOPLANKTON PRODUCTION AND

TRANSPORT OF PHOTOSYNTHETIC

CARBON TO BACTERIA

WILEY-INTERSCIENCE



PHYTOPLANKTON PRODUCTION AND TRANSPORT OF PHOTOSYNTHETIC
CARBON TO BACTERIA

Michael F. Coveney

The dissertation is a summary of the following published
and unpublished papers:

- I. Phytoplankton, zooplankton and bacteria - standing crop
and production relationships in a eutrophic lake. *Oikos*
29:5-21. 1977. (co-authors: G. Cronberg, M. Enell, K.
Larsson and L. Olofsson).
- II. Photosynthetic capacity and growth rate constants for
Lake Bysjön phytoplankton, 1975.
- III. Separation of algae and bacteria in lake water by size
fractionation. *Verh. Internat. Verein. Limnol.* 20:1264-
1269. 1978.
- IV. Fluorometric determination of chlorophyll *a* and pheopig-
ment *a* without chlorophyll *b* interference.
- V. Transport of photosynthetic carbon from phytoplankton to
bacteria in freshwater plankton communities.

The above Roman numerals will be used when reference is made
to the papers in the summary.

Introduction

Most of the organic carbon found in natural freshwaters is in dissolved form, commonly 85 to 90% of the total organic carbon present (Wetzel 1975). Dissolved organic substances in aquatic ecosystems are subject to numerous biotic and abiotic transformations. A large part of the total organic carbon pool is biologically inactive, however. Changes in concentrations of labile substances are superimposed on this more stable background.

Two processes can be distinguished which contribute to the autochthonous dissolved organic matter in aquatic systems: decomposition of dead plants and animals and release of organic matter from living organisms. In particular, release of organic carbon from phytoplankton has received a great deal of attention (reviews in Fogg 1971, Hellebust 1974). The accuracy of much of the earlier work has been questioned on methodological grounds, however (Sharp 1977).

The ecological significance of extracellular release from phytoplankton can be seen in two perspectives. First, it is an element of pelagic carbon cycling. Organic carbon is released from phytoplankton and made available to bacteria. Here, the size of the flux is important - the amount of carbon lost from the algae and the amount available to the bacteria.

Secondly, substances can be released which have specific effects on the metabolism of other organisms (Fogg 1971). Since these substances can be active at low concentrations,

the important factor is not the amount of carbon released, but its biological effects. Investigations of these two aspects of phytoplankton extracellular release are not mutually exclusive in theory, but are often so in practice because of the different methods required.

I studied extracellular release from phytoplankton as part of carbon cycling in a small, eutrophic lake in south Sweden. H^{14}CO_3 tracer techniques were used to measure phytoplankton productivity and to study transport of carbon from phytoplankton to dissolved and small particulate pools. Initially, phytoplankton ^{14}C productivity and net extracellular release were measured *in situ* during a growth season. These data were used in a semi-quantitative study of phytoplankton growth and of relationships to zooplankton and bacteria (I, II).

Investigation of heterotrophic uptake of algal products required modification of existing methodology, including 1) evaluation of size fractionation methods for separation of algae and bacteria in lake water (III), and 2) improvement in fluorometric methods for determination of chlorophyll *a* (IV). These modified methods were then used in ^{14}C tracer experiments *in situ* and in the laboratory to study short-term bacterial uptake of algal extracellular products (IV).

The investigations were made in the hard-water Lake By-sjön in south Sweden (I). Enell (1980) treated water and sediment chemistry of the lake, and a description of the littoral fauna is found in Herrmann (1979).

Methodological and other details and most references to

the literature are omitted in the summary; this information is found in the five papers discussed.

Methods Development (III, IV)

Differentiation between ^{14}C in algae and that in bacteria after incubation of natural samples with H^{14}CO_3 was necessary for study of bacterial uptake of algal extracellular products. I tested size fractionation as a method for separation of the two organism groups and found it to function satisfactorily with Lake Bysjön plankton (III). From 50 to 85% of the bacterial cells in a 20 ml water sample passed through a Whatman GF/C filter, together with only 0.04 to 1.4% of the chlorophyll a (III, V). Separation of planktonic algae and bacteria was better with a GF/C filter than with a 3 μm Nuclepore filter, although the size of particles passing through the GF/C filter is not as well-defined (III).

Successful use of size fractionation methods required determination of algal biomass passing through the fractionating filter for each experiment. Because photosynthetic activity was measured, chlorophyll a was the biomass parameter monitored. Fluorometric determination of extracted chlorophyll a gave the required precision and low detection limit. The standard fluorometric method is subject to interference from pheophytinization of chlorophyll b , however, which can result in serious underestimation of chlorophyll a . A method was developed which utilizes the difference in pheophytinization rates of chlorophylls a and b to achieve a kinetic separation of the two pigments (IV). The modified procedure eliminates chlorophyll b interference in determination of chlorophyll a and reduces interference from chlorophyll $c_1 + c_2$. In devel-

oping the method, I found that the pheophytinization reaction is second order with respect to H^+ , instead of first order as frequently cited. This same conclusion had been reached earlier in an apparently overlooked PhD dissertation (Cho 1966).

Photosynthetic Capacity, Growth Rate Constants and Extracellular Release of Phytoplankton in Lake Bysjön, 1975 (I, II)

Seasonal variation in phytoplankton biomass in Lake Bysjön was dominated by two maxima during 1975, *Stephanodiscus hantzschii* Grun. in spring and *Aphanizomenon flos-aquae* Ralfs in summer. Corresponding chlorophyll *a* concentrations in the mixed layer were 170 and 130 mg·m⁻³, respectively. A period of low phytoplankton biomass (ca. 1 mg chlorophyll *a*·m⁻³) persisted from the end of April until development of green algae in June.

Photosynthetic capacity ($P_{\max}$) for Lake Bysjön phytoplankton varied between 0.70 and 11 mg C·mg chl *a*⁻¹·h⁻¹ during 1975. Relationships between log $P_{\max}$ and temperature, chlorophyll *a*, O₂ concentration, O₂ % saturation and pH were tested using regression analysis. Of the single independent variables, regression on temperature had the highest r^2 , 0.441. With temperature as the first independent variable, multiple regression with chlorophyll *a* as the second independent variable explained the most remaining variance (r^2 0.687).

The positive relationship between community $P_{\max}$ and temperature is often seen in freshwater systems and can have both physiological and successional causes. The negative relationship with phytoplankton biomass (chlorophyll *a*) is also common, but the causes are likely to be complex. In Lake Bysjön, nutrient (Si, N) limitation probably caused low $P_{\max}$ at peak *Stephanodiscus* biomass and during summer green algal development.

Growth rate constants for phytoplankton were calculated from *in situ* ^{14}C productivity measurements. Most rate constants ranged from 0.05 to 0.60 d^{-1} (mean values for mixed layer), with three values over 1.0 d^{-1} during the low biomass period. There was a distinct inverse relationship with phytoplankton chlorophyll *a*.

Growth rate constants during build-up and decline of the spring *Stephanodiscus* maximum were compared with changes in phytoplankton carbon standing crop in the lake and with estimated grazing losses. Total losses (net productivity per day minus change in standing crop) were consistently greater than grazing losses, which indicated the importance of death and/or sedimentation processes. A postulated mean sinking rate of $0.56\text{ m}\cdot\text{d}^{-1}$ was necessary to account for standing crop losses during the *Stephanodiscus* maximum.

Net extracellular release from phytoplankton during 4 h incubation was low in Lake Bysjön during 1975 - generally under 1% of carbon assimilation per square meter lake surface. Maximal bacterial numbers occurred immediately after the two phytoplankton biomass maxima. This showed the importance of algal decomposition products, rather than excretion products, as substrates for peak bacterial development. It could not be concluded, however, that gross extracellular release was also low, since heterotrophic uptake would remove ^{14}C -labeled products during incubation. In addition, delay in attainment of intracellular $^{14}\text{C}/^{12}\text{C}$ equilibria could also have caused underestimation of extracellular release.

Transport of Photosynthetic Carbon from Phytoplankton to
Bacteria (V)

Size fractionation methods were used in laboratory and *in situ* H^{14}CO_3 tracer experiments to study movement of photosynthetic carbon from phytoplankton to dissolved material and small particles. ^{14}C fixed in photosynthesis was recovered in dissolved and small particulate fractions. Although other interpretations are possible, the small particulate ^{14}C probably represented bacterial uptake of algal extracellular products.

Gross *in situ* extracellular release (dissolved + small particulate ^{14}C) was maximal at or near the surface. Release as a percent of total carbon assimilation was usually minimal at the depth of peak algal productivity. Extracellular release was from 1.2 to 6.8% of total productivity per square meter lake surface. From 36 to 73% of this was found in the small particulate fraction after ca. 4 h, indicating the importance of short-term transport to small particles. Despite inclusion of the small particulate ^{14}C , however, percent release was low compared to many reports in the literature.

The percent of algal extracellular ^{14}C transported to small particles was relatively constant within each vertical profile. Small particulate ^{14}C uptake per square meter lake surface varied little throughout the experiments, but dissolved ^{14}C was inversely related to water temperature.

Kinetics of ^{14}C accumulation in dissolved and small particulate fractions was investigated at constant light and *in*

situ temperature in the laboratory. One time-series experiment showed patterns of ^{14}C increase consistent with a steady state pool of algal extracellular products. The remaining two experiments showed almost linear increase in dissolved ^{14}C during 5.5 h, and this pattern was most consistent with increasing pool size. Since the extracellular products of algae do not accumulate over long time periods *in situ*, an increasing pool size in the light should be balanced by a decrease in the dark, resulting in diel fluctuation in concentration.

The calculation of bacterial production from algal excretion products using size fractionation data requires assumptions concerning isotopic equilibria in the different carbon pools, the precise nature of small particulate ^{14}C , bacterial respiration of assimilated carbon and excretion behavior during a 24-h light/dark cycle. Data from Lake Bysjön were not sufficient to support all the necessary assumptions. Instead, ^{14}C accumulation in small particles was taken as a minimal estimate of net bacterial production from algal products during incubation. This was 32 to 95% of total heterotrophic bacterial production (estimated as dark $^{14}\text{CO}_2$ uptake) on four occasions. Thus, while excretion was apparently not an important carbon loss for phytoplankton, it may have represented an important carbon source for planktonic bacteria.

Acknowledgements

I thank Prof. S. Björk and Dr. A. Södergren for supporting the different phases of my work and for reading and criticizing the manuscripts. Dr. B. Riemann, Dr. B. Hargrave, Dr. J. F. Talling, Dr. G. Andersson and Dr. H.L. Allen read parts of the manuscripts and suggested numerous improvements. I also thank Prof. C. Weibull and Prof. L.O. Björn for discussions of sections of the work and for use of instrumentation. Prof. S. Petterson provided LSC facilities and helpful advice.

The assistance and data of other members of the Lake By-sjön project are gratefully acknowledged. G. Jönsson kindly did much of the typing.

Portions of this work were supported by the Swedish Natural Science Research Council (NFR), the National Swedish Environment Protection Board (SNV) and the Royal Physiographic Society of Lund.

The dissertation is dedicated to my parents, Dorothy P. Coveney and Erwin L. Coveney, with affection and in appreciation.

References

- Cho, D.H. 1966. Kinetics of the acid catalyzed pheophytinization of chlorophylls. Ph.D. Diss. Univ. of California, Davis. 86 p.
- Enell, M. 1980. The phosphorus economy of a hypertrophic seepage lake in Scania, south Sweden. Ph.D. Diss. Univ. of Lund. 190 p.

- Fogg, G.E. 1971. Extracellular products of algae in fresh-water. *Ergebn. Limnol.* 5:1-25.
- Hellebust, J.A. 1974. Extracellular products. In: *Algal physiology and biochemistry* (Ed. W.D.P. Stewart). Botan. Monogr. 10. Blackwell, Oxford. 989 p.
- Herrmann, J. 1979. Population dynamics of *Dendrocoelum lacteum* (O.F. Müller) (Turbellaria, Tricladida) in a South Swedish lake. *Arch. Hydrobiol.* 85:482-510.
- Sharp, J.H. 1977. Excretion of organic matter by marine phytoplankton: Do healthy cells do it? *Limnol. Oceanogr.* 22: 381-399.
- Wetzel, R.G. 1975. *Limnology*. W.B. Saunders Co. Philadelphia. 743 p.

THE UNIVERSITY OF CHICAGO
LIBRARY

1000 S. EAST ASIAN AVENUE
CHICAGO, ILL. 60607

THE UNIVERSITY OF CHICAGO
LIBRARY

1000 S. EAST ASIAN AVENUE
CHICAGO, ILL. 60607

1000 S. EAST ASIAN AVENUE

Photosynthetic Capacity and Growth Rate Constants
for Lake Bysjön Phytoplankton, 1975

Michael F. Coveney

Institute of Limnology
Box 3060
S-220 03 Lund, Sweden



1980

CONTENTS

	<u>Page</u>
Abstract	1
Introduction	2
Methods and Materials	3
Results and Discussion	5
References	11
Tables	13
Figures	14



ABSTRACT

As part of a broad study of the planktonic community, phytoplankton ^{14}C productivity and chlorophyll a were determined during 1975 in the small eutrophic Lake Bysjön, south Sweden. Seasonal changes in photosynthetic capacity (P_{max}) and in growth rate constants for phytoplankton were compared with variations in phytoplankton density and in physical and chemical factors. Phytoplankton chlorophyll a showed two dominating maxima during the growth season: *Stephanodiscus hantzschii* Grun. in spring and *Aphanizomenon flos-aquae* Ralfs in summer. Phytoplankton growth rate constants were low (ca. 0.05 d^{-1}) at biomass maxima and increased to over 1.0 d^{-1} during a low biomass period. P_{max} ranged from 0.70 to 11 mg C mg chl $a^{-1} \text{ h}^{-1}$. On a seasonal basis, P_{max} varied positively with temperature and negatively with chlorophyll a (and correlates of chlorophyll a). At least for certain periods, depression of P_{max} was apparently due to nutrient (Si, N) limitation.

INTRODUCTION

Lake Bysjön, a small eutrophic lake in south Sweden, was the object of a broad study of relationships among planktonic organisms and water chemistry in 1975 (Coveney et al. 1977). As part of that study, growth rate constants were estimated for phytoplankton during the spring diatom maximum and compared with biomass changes in the lake. The work presented here extends sections of that study to include seasonal changes in phytoplankton photosynthetic capacity ($P_{\max}$) and estimates of growth rate constants over the entire growing season. These are compared with changes in phytoplankton density and in nutrients and physical factors. Dr. M. Enell provided the water chemical data, and Dr. G. Cronberg made the quantitative phytoplankton analyses. Dr. J.F. Talling kindly read and criticized an earlier version of the manuscript.

METHODS AND MATERIALS

Water analyses and determination of phytoplankton ^{14}C productivity were done at one to two week intervals from January to November, 1975, a total of ca. 23 sampling occasions. ^{14}C productivity was determined with ca. 4 h *in situ* exposures at midday. Daily productivity was calculated from the midday integral value using the relationship (irradiance per day)/(irradiance per exposure). Phytoplankton carbon standing crop was determined from direct counts and cell volume estimates using the equations of Strathmann (1967).

Mean rate constants (k_1) for phytoplankton growth in the mixed layer were calculated from phytoplankton productivity and carbon standing crop as described in Coveney et al. (1977). This calculation requires the following simplifying assumptions: 1) Phytoplankton biomass is evenly distributed in the mixed layer; 2) ^{14}C methodology measures net productivity; and 3) Phytoplankton respiration is equal to 10% of gross productivity at optimal irradiance and is constant over the 24-h day. Night length was from 7 to 13 h for the period considered. Sampling and analytical procedures for phytoplankton carbon, water chemistry parameters and phytoplankton productivity are described in more detail in Coveney et al. (1977).

Water samples for chlorophyll *a* determination were filtered through Whatman GF/C filters, which were stored desiccated under vacuum at -20 C until analysis. Phytoplankton pigments were extracted from ground filters with 90% aqueous acetone (neutralized with MgCO_3) for 24 h in the dark at 4 C. Chloro-

phyll α (corrected for pheopigments) was determined in centrifuged extracts according to Lorenzen (1967).

Unless otherwise noted, results of biological and chemical analyses are expressed as mean values for the mixed layer. Lake Bysjön is often stratified during summer, but temperature rarely follows the classic pattern with depth due to warming of the hypolimnion and occasional temporary thermal stratification in the upper few meters. For this reason, vertical variations in both temperature and O_2 were used to judge the depth of the primary mixed layer. The mean value of a given parameter (either per m^2 or per m^3) for this mixed layer was calculated using a horizontal weighting scheme based on basin morphometry. Mean pH was calculated from mean H^+ concentration.

The following symbols are used:

- P_{max} photosynthetic capacity, carbon assimilation per unit chlorophyll α at light saturation. Unit: $mgC \cdot mg\ chl\ \alpha^{-1}\ h^{-1}$.
- k_1 rate constant for phytoplankton growth based on ^{14}C uptake. Unit: d^{-1} .
- Z_m depth of the primary mixed layer. Unit: m.

RESULTS AND DISCUSSION

Phytoplankton biomass in Lake Bysjön during 1975 was dominated by two maxima, *Stephanodiscus hantzschii* Grun. in spring and *Aphanizomenon flos-aquae* Ralfs in summer. *Sphaerocystis schroeteri* Chod. and *Volvox aureus* Ehrb. dominated just before buildup of the *Aphanizomenon* bloom, and *Melosira granulata* var. *angustissima* Müller had a short maximum at the end of August (Coveney et al. 1977).

Maximum phytoplankton productivity values of $2.5 \text{ g C m}^{-2} \text{ d}^{-1}$ and $5.0 \text{ g C m}^{-2} \text{ d}^{-1}$, respectively, were measured just before peak *Stephanodiscus* biomass and at the peak *Aphanizomenon* biomass development (Fig. 1). The corresponding maximal chlorophyll *a* concentrations were 170 mg m^{-3} and 130 mg m^{-3} (mean values for mixed layer). Low phytoplankton biomass levels (under $1 \text{ mg chl } a \text{ m}^{-3}$) were present from the end of April until development of green algae in June (Fig. 1). The strongly bimodal seasonal pattern of phytoplankton biomass observed in 1975 is characteristic for Lake Bysjön, although timing and species composition can vary from year to year (Cronberg and Enell, unpublished data). Explanations for the early summer period with unusually low biomass were discussed by Coveney et al. (1977).

In a system with such intense fluctuations in algal productivity, simultaneous effects on the physical and chemical environment (e.g. inorganic nutrients, O_2 , pH) are expected, and these were observed in Lake Bysjön (Fig. 2, 3). Both dissolved oxygen and pH rose sharply during the spring and summer phytoplankton peaks and decreased during the intervening low biomass

period (Fig. 2). Surface concentrations of reactive silica decreased rapidly from about 3.0 to 0.02 mg SiO₂ l⁻¹ during the spring *Stephanodiscus* development. Silica increased during the summer and was again depleted during the short *Melosira* peak in August. Inorganic N concentrations decreased during the spring diatom peak from more than 2000 to about 600 µg N l⁻¹ and dropped to about 50 µg l⁻¹ just before *Aphanizomenon* development in June. While PO₄-P also fluctuated with phytoplankton development, the high concentrations found in Lake Bysjön surface water were never seriously depleted (minimum 170 µg l⁻¹) (Fig. 3).

Daily productivity values were calculated from short-term exposures using the irradiance curve method. This assumes a linear relationship between integral productivity and surface irradiance, whereas the relationship is nearly logarithmic because of light saturation of photosynthesis (Talling 1957). For incubations at midday (i.e. at highest irradiance levels) the irradiance curve method gives the same or lower daily productivity than more accurate models, e.g. that of Talling (1957). The productivity values used here are therefore minimal estimates.

The majority of ¹⁴C growth rate constants calculated for the mixed layer ranged from 0.05 to 0.60 d⁻¹, with three values above 1.0 d⁻¹ (Fig. 4). There was a distinct inverse relationship with phytoplankton chlorophyll *a* (Fig. 1, 4). The lowest rate constants corresponded to the two algal biomass maxima, and high values were found during the low biomass period after decline of the spring diatom development.

Some seasonal correspondence was seen between ^{14}C growth rate constants and P_{max} ; for example, the low rate constants at peak *Stephanodiscus* biomass in spring were a direct result of decreased P_{max} (Fig. 4). As discussed by Eppley (1972), P_{max} can be related to a growth rate constant calculated for a unit water volume if the ratio algal carbon/chl a is known. Any relationship for the present data is more complex, however, since the growth rate constants represent mean values for the mixed layer where photosynthesis in much of the water volume is light-limited.

P_{max} varied between 0.70 and 11 mg C·mg chl $a^{-1}\text{h}^{-1}$. The values showed a rising trend with temperature during the summer and were low during both peak biomass periods (Fig. 4). Relationships between P_{max} and temperature, chlorophyll a , dissolved O_2 concentration, dissolved O_2 saturation and pH in the mixed layer, both singly and in combination, were tested using regression analysis. Of the five possible regressions with single independent variables, significant relationships were found for temperature, O_2 concentration and chlorophyll a (Table 1). Temperature (highest r^2 , 0.441) was taken as the first independent variable, and regression equations for two independent variables were calculated with each of the remaining parameters. Only chlorophyll a and pH showed significant regression coefficients (negative), and chlorophyll a gave the higher r^2 value, 0.687 (Table 1). A further addition of variables gave no significant regression coefficients.

The importance of temperature effects on community P_{max} is well-established for inland waters (reviewed in Harris

(1978)). Eppley (1972) concluded that other factors, especially nutrient limitation, have resulted in relatively few oceanic investigations giving the expected relationship. The regression equation for the Lake Bysjön data gave a Q_{10} for $P_{\max}$ of 1.9, comparable with other published values (Harris 1978).

Chlorophyll *a* as the second variable giving the highest r^2 in the multiple regression supports the observation of depressed $P_{\max}$ at high biomass levels (Fig. 4), but reveals nothing about the mechanism. Bindloss (1974) discussed nutrient depletion, shade adaptation, increased pH and increased O_2 as possible reasons for lower $P_{\max}$ at high phytoplankton densities. No evidence for shade adaptation (high cell chlorophyll *a* content, saturation of photosynthesis at low irradiance) was seen in Lake Bysjön during the spring *Stephanodiscus* peak (data not shown). Both pH and O_2 were inferior to chlorophyll *a* in explaining remaining variance in $P_{\max}$ after temperature effects (Table 1), although pH also had a significant, negative regression coefficient.

Bindloss (1974) concluded that pH values above ca. 9.0 reduced $P_{\max}$ for phytoplankton in Loch Leven. Jones (1978) found depression of $P_{\max}$ above pH ca. 9.5 in Lough Neagh. While pH was high in Lake Bysjön surface water in connection with maximal chlorophyll *a* (pH 9.3 to 9.6, Fig. 2), total alkalinity was also high (not under 1.9 meq l^{-1} during 1975). Carbon limitation should not have occurred for any alga capable of utilizing bicarbonate.

Depression of $P_{\max}$ at high biomass levels could have been an artifact resulting from enclosure of rapidly photo-

synthesizing algae for the ca. 4 h *in situ* exposures. At peak *Stephanodiscus* biomass, however, O_2 in the incubation bottle would have theoretically increased only 1.8 mg l^{-1} (14 %-saturation units) and pH 0.1 unit, even if community respiration is ignored (P.Q. 1.0, pH changes calculated from tables in Rebsdorf (1972)). Theoretical changes in the incubation vessel at minimal $P_{\max}$ during the *Aphanizomenon* bloom were higher, $4.7 \text{ mg } O_2 \text{ l}^{-1}$ (52 %-saturation units) and 0.2 pH unit. The degree to which these values exceeded the changes in the same parameters occurring simultaneously in the lake is not known, however.

Bindloss (1974) found that the photosynthetic capacity of Loch Leven phytoplankton did not begin to decline until exposures exceeded 6 h. This was in a lake with alkalinity 1.0 to 1.6 meq l^{-1} and where daily productivity values equal to the peak values from Lake Bysjön are often recorded. In addition, low $P_{\max}$ at high population densities has been observed even with shorter incubation times (Gelin 1975, Jones 1977).

While the possibility of incubation artifacts cannot be excluded, nutrient limitation is a more likely explanation for the apparent inverse relationship between chlorophyll *a* and $P_{\max}$. Low $P_{\max}$ at the spring *Stephanodiscus* maximum was probably a result of Si limitation, as reactive Si decreased to $0.02 \text{ mg SiO}_2 \text{ l}^{-1}$ in surface water at the biomass peak (Fig. 3). $P_{\max}$ (and k_1) increased immediately afterwards as dominance shifted to cryptomonads (Coveney et al. 1977), although a decrease at the beginning of May has no obvious ex-

planation. Depletion of inorganic N during green algal dominance in June - July probably caused the simultaneous drop in $P_{\max}$. $P_{\max}$ increased somewhat during the subsequent *Aphanizomenon* bloom (Fig. 4), but high O_2 levels may have maintained the generally low $P_{\max}$ values by enhancing photorespiration (Fogg et al. 1973). Thus, while chlorophyll *a* was the best correlate with depressed $P_{\max}$ over the entire growth season, different causal mechanisms probably operated for the different algal communities present.

REFERENCES

- Bindloss, M.E. 1974. Primary productivity of phytoplankton in Loch Leven, Kinross. *Proc. R. Soc. Edinb. B* 74:157-181.
- Coveney, M.F., G. Cronberg, M. Enell, K. Larsson, & L. Olofsson. 1977. Phytoplankton, zooplankton and bacteria - standing crop and production relationships in a eutrophic lake. *Oikos* 29:5-21.
- Eppley, R.W. 1972. Temperature and phytoplankton growth in the sea. *Fish. Bull.* 70:1063-1085.
- Fogg, G.E., W.D.P. Stewart, P. Fay & A.E. Walsby. 1973. *The blue-green algae*. Academic Press. N.Y.
- Gelin, C. 1975. Nutrients, biomass and primary productivity of nannoplankton in eutrophic Lake Vombsjön, Sweden. *Oikos* 26:121-139.
- Harris, G.P. 1978. Photosynthesis, productivity and growth: The physiological ecology of phytoplankton. *Ergebn. Limnol.* 10:I-IV, 1-171.
- Jones, R.I. 1978. Adaptations to fluctuating irradiance by natural phytoplankton communities. *Limnol. Oceanogr.* 23: 920-926.
- Jones, R.I. 1977. Factors controlling phytoplankton production and succession in a highly eutrophic lake (Kin-nego Bay, Lough Neagh). II. **Phytoplankton** production and its chief determinants. *J. Ecol.* 65:561-577.
- Lorenzen, C.J. 1967. Determination of chlorophyll and pheo-pigments: spectrophotometric equations. *Limnol. Oceanogr.* 12:343-346.

- Rebsdorf, A. 1972. The carbon dioxide system in freshwater. Freshwater Biological Laboratory, Hillerød, Denmark.
- Strathmann, R.R. 1967. Estimating the organic carbon content of phytoplankton from cell volume or plasma volume. Limnol. Oceanogr. 12:411-418.
- Talling, J.F. 1957. The phytoplankton population as a compound photosynthetic system. New Phytol. 56:133-149.

Table 1. Regression analysis for Lake Bysjön data with $\log P_{\max}$ as dependent variable. A) Regression with single independent variable. B) Multiple regression with temperature as first independent variable (X_1). Significance of regression coefficients determined with F-test. $n = 20$.

A.	Y	X	r^2	Regression coeff.		
				sign	F	p<
	$\log P_{\max}$	temperature	0.441	+	14.2	0.005
		O ₂ conc.	0.379	-	11.0	0.005
		chl α	0.360	-	10.1	0.01
		O ₂ % sat.	0.129	-	2.7	ns
		pH	0.043	-	0.8	ns

B.	Y	X_1	X_2	r^2	X_2 regression coeff.		
					sign	F	p<
	$\log P_{\max}$	temperature	chl α	0.687	-	13.3	0.005
			pH	0.616	-	7.7	0.025
			O ₂	0.524	-	2.9	ns
			O ₂ %	0.511	-	2.4	ns

ns: not significant, $p > 0.05$

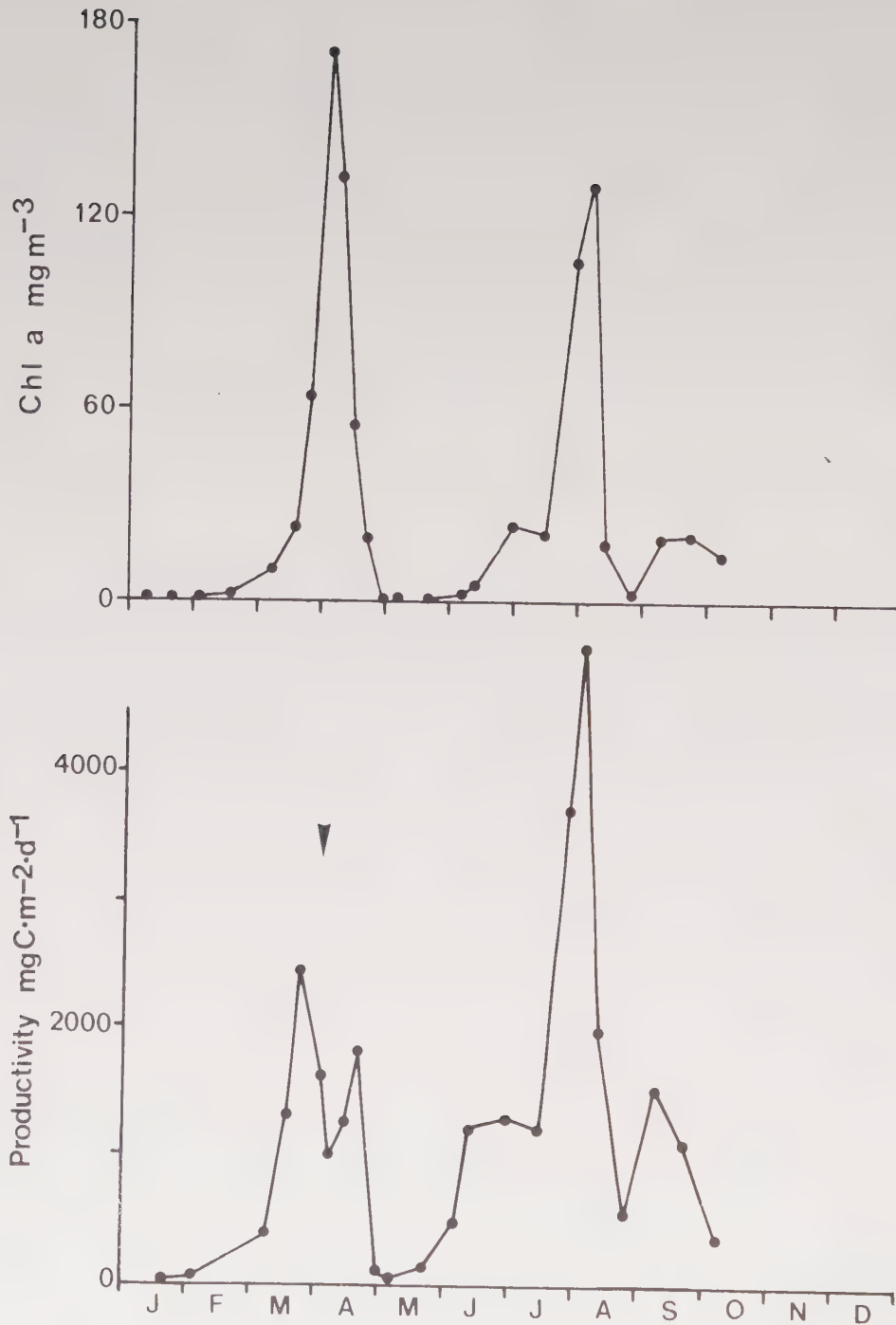


Figure 1. Lake Bysjön, 1975. Mean chlorophyll *a* concentration in mixed layer and phytoplankton ¹⁴C productivity. Arrow indicates spring phytoplankton biomass maximum.

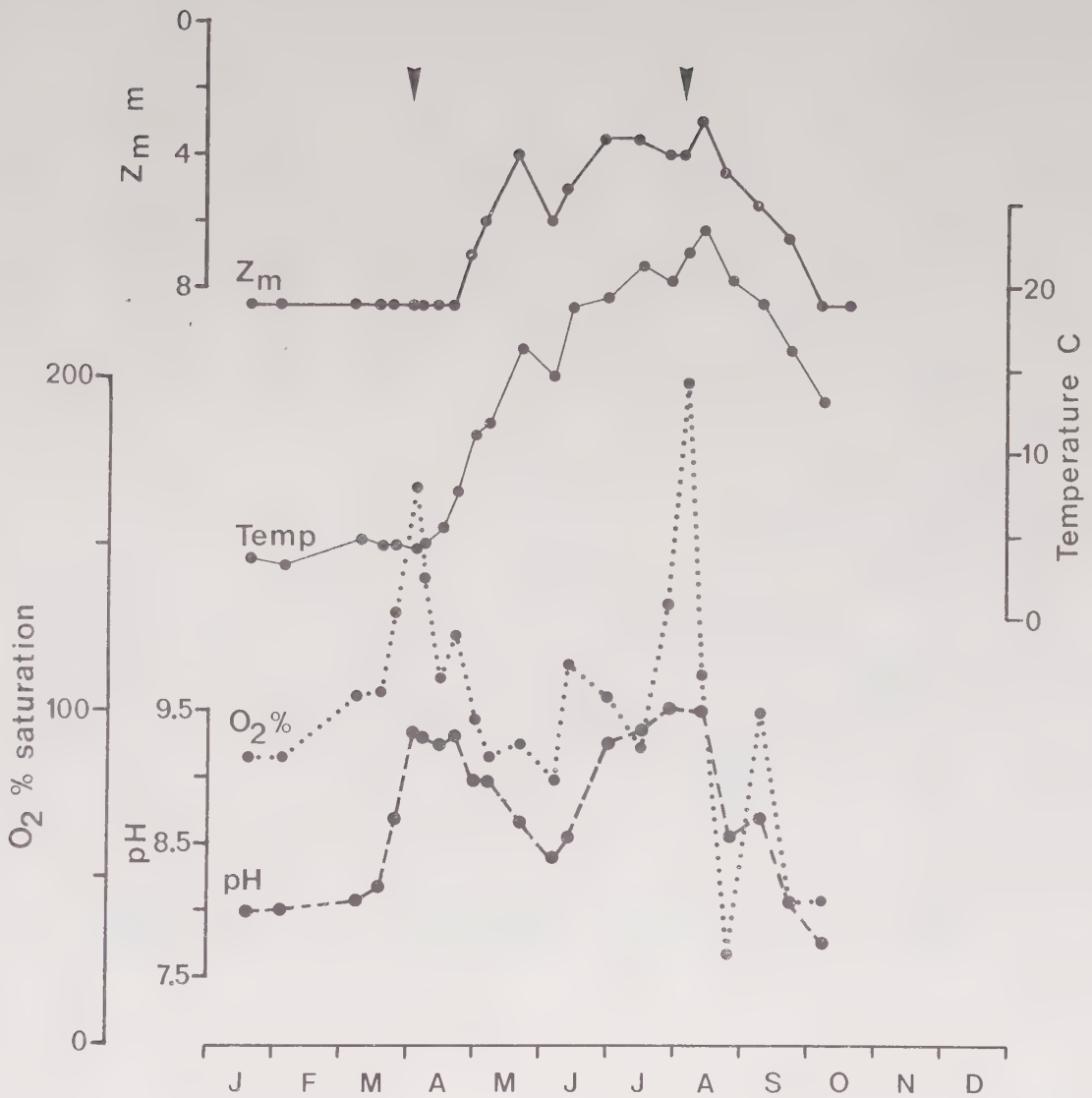


Figure 2. Lake Bysjön, 1975. Depth of mixed layer (Z_m) and mean temperature, O_2 saturation and pH in mixed layer. Arrows indicate phytoplankton biomass maxima.

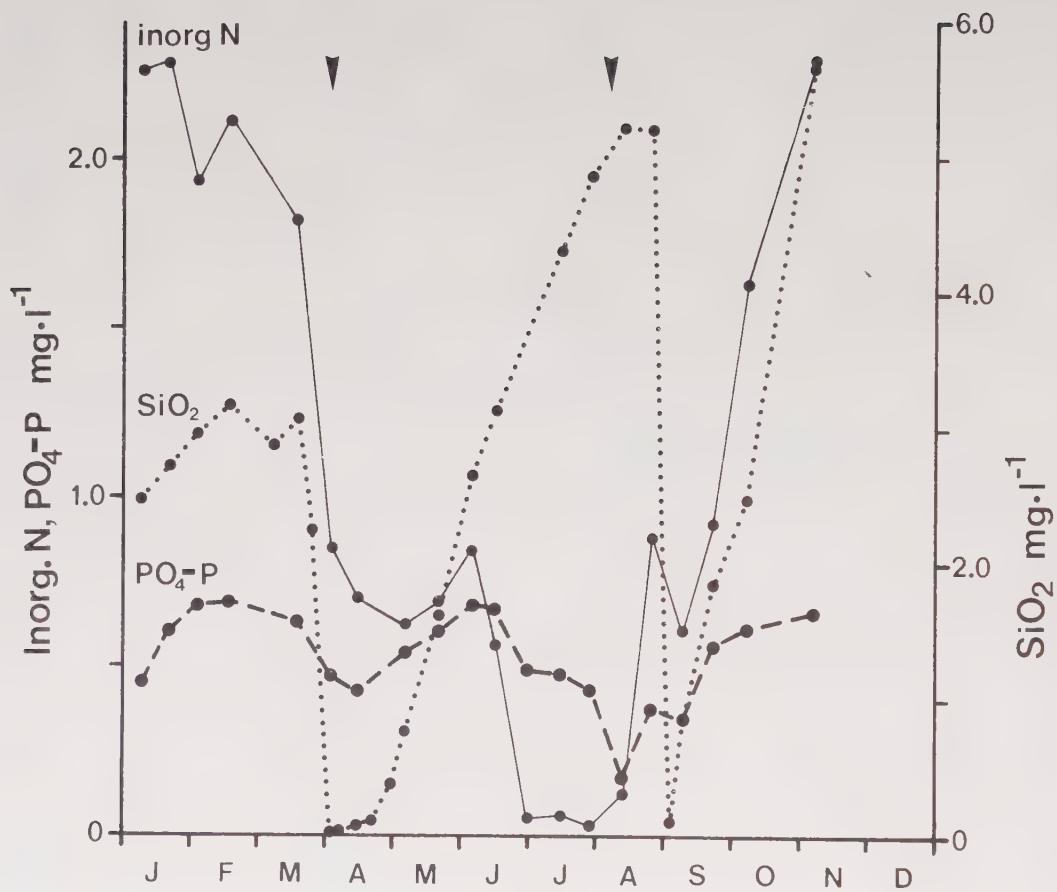


Figure 3. Lake Bysjön, 1975. Concentrations at 1.0 m of inorganic N, PO₄-P and SiO₂. Arrows indicate phytoplankton biomass maxima.

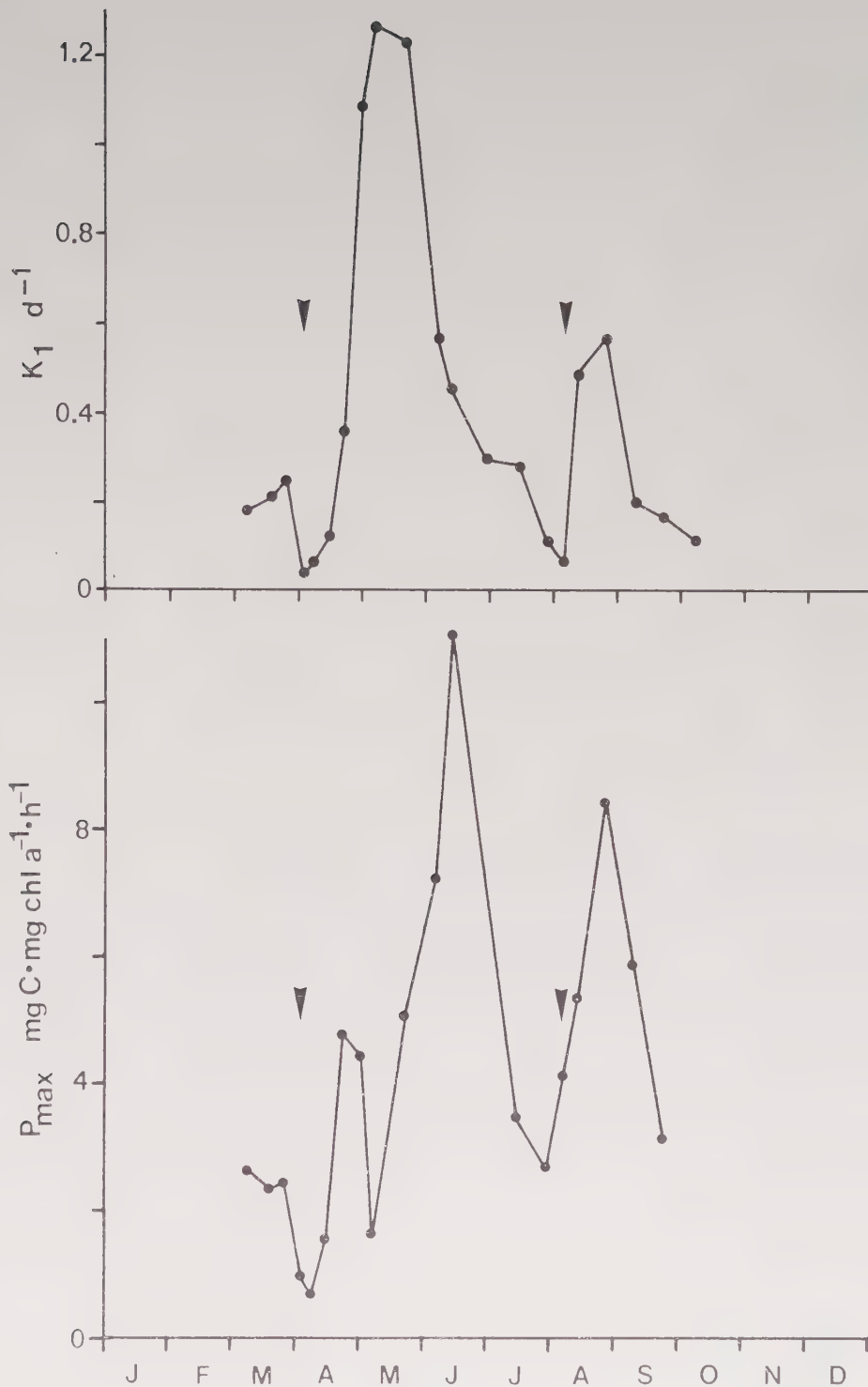


Figure 4. Lake Bysjön, 1975. Mean phytoplankton growth rate constant for mixed layer (k_1) and photosynthetic capacity (P_{max}). Single determinations in January and February are omitted, and two additional points are omitted for P_{max} because light-saturated rates were not attained. Arrows indicate phytoplankton biomass maxima.

Fluorometric Determination of Chlorophyll *a*
and Pheopigment *a* without Chlorophyll *b*
Interference

Michael F. Coveney

Institute of Limnology
Box 3060
S-220 03 Lund, Sweden



1980

CONTENTS

	<u>Page</u>
Abstract	1
Introduction	2
Methods and Materials	4
Results and Discussion	11
Acknowledgements	17
References	18
Tables	21
Figures	25

ABSTRACT

A single-wavelength acidification method is presented for fluorometric determination of chlorophyll *a* and pheopigment *a* in 90% acetone extracts. The method utilizes the different pheophytinization rates of chlorophylls *a* and *b* to eliminate interference due to chlorophyll *b* in natural samples. Compared to standard methods, chlorophyll *c* interference is also decreased in determination of chlorophyll *a*, but is increased for pheopigment *a*. When chlorophyll *b* is present at high levels (molar ratio chl *b/a* > ca. 0.2), it can also be determined with no extra measurements. The modified procedures are only slightly more complicated than standard methods and do not change analytical precision for chlorophyll *a*.

INTRODUCTION

The fluorometric determination of chlorophyll *a* extracted from natural phytoplankton communities is not specific. In addition to chlorophyll *a* degradation products, chlorophylls *b* and *c* and their pheopigments fluoresce in the same region as chlorophyll *a*. Chlorophyll *b* and *c* interference is especially acute when acidification techniques are used to estimate chlorophyll *a* and pheopigment *a*. The extent of this interference was shown by Loftus & Carpenter (1971), who also described a fluorometric method for determining chlorophylls *a*, *b* and *c* and pheopigment *a*. Their procedure requires knowledge of 18 conditional fluorescence coefficients, however, and six measurements on each sample. As shown by Holm-Hansen et al. (1965), chlorophyll *c* interference can be largely eliminated by emission filters. The most significant remaining interference in determination of chlorophyll *a* and pheopigment *a* using acidification techniques is from the simultaneous conversion of chlorophyll *b* to pheophytin *b*. A single wavelength acidification method, requiring only four molar fluorescence coefficients, is described here which eliminates this problem.

The method is based on the procedure of Holm-Hansen et al. (1965), but makes use of the fact that pheophytinization is more rapid for chlorophyll *a* than for chlorophyll *b* under acidic conditions in acetone (Mackinney & Joslyn 1940; Schanderl et al. 1962). Because of similar fluorescence characteristics (Loftus & Carpenter 1971), chlorophyllide *a* is measured as chlorophyll *a*, and pheophorbide and pheophytin *a*

are quantified together as pheopigments. Distinction of the free acids from the phytol esters requires physical separation, e.g. phase separation (Whitney & Darley 1979) or chromatography (Abaychi & Riley 1979). As discussed by Loftus & Carpenter (1971), fluorescence from substances other than chlorophylls and their degradation products at the excitation and emission wavelengths used here does not appear to be important in extracts from natural algal communities.

METHODS AND MATERIALS

When fluorescence is measured at the chlorophyll *a* excitation and emission maxima, pheophytin *a* fluoresces less, and pheophytin *b* more, than the respective chlorophylls. If an extract containing the two chlorophylls is acidified, fluorescence drops rapidly due to conversion of chlorophyll *a* to pheophytin *a* and then slowly rises to a final equilibrium due to continued conversion of chlorophyll *b* (Fig. 1).

Because of this difference in pheophytinization rate, a kinetic separation of the two chlorophylls in a crude extract can be achieved as follows: The initial fluorescence of the extract is measured at chlorophyll *a* excitation and emission maxima. A precise, rapid addition of HCl is made to the cuvette at time 0, and conversion of chlorophyll *a* proceeds to completion during time T while a known fraction of the chlorophyll *b* present is converted. A second measurement of fluorescence is made at the same wavelengths at time T, after which a stronger HCl solution is added to complete conversion of chlorophyll *b*. This is followed by a third measurement of fluorescence at equilibrium. Molar concentrations of chlorophylls *a* and *b* and pheopigment *a* in the extract can be calculated from these three fluorescence values and molar fluorescence coefficients for chlorophylls *a* and *b* and their pheophytins. Optimal reaction time T, where pheophytinization of chlorophyll *a* is just complete, is calculated from the chlorophyll *a* reaction rate constant. The rate constant for chlorophyll *b* pheophytinization is also needed to calculate the fraction chlorophyll *b* converted at time T.

As an alternative, only the first two measurements of fluorescence (initial, time T) are made. Chlorophyll and pheopigment *a* can then be estimated with reduced interference from chlorophyll *b* compared to standard methods which usually involve acidification to equilibrium. These methods are also applicable to spectrophotometric measurements involving acidification, although chlorophyll *b* interference is less serious there (Marker 1972).

The following equations for calculation of chlorophyll *a* and *b* and pheopigment *a* concentrations were derived assuming that the measurements of fluorescence represent the sum of fluorescence of these three components (unacidified) or their acidification products (acidified) without interactions. In addition, a fourth component X was included in expressions used to predict the effect of any other substance on the measurements if the fluorescence behavior of the substance is known.

$$M_b = \frac{F^e - F^h}{\gamma_{pb} - \gamma_b^h} \quad (1)$$

$$E_b = \frac{X^e - X^h}{\gamma_{pb} - \gamma_b^h} \quad (2)$$

$$M_a = \frac{F^u - F^h - M_b(\gamma_b^u - \gamma_b^h)}{\gamma_a - \gamma_{pa}} \quad (3)$$

$$E_a = \frac{X^u - X^h - E_b(\gamma_b^u - \gamma_b^h)}{\gamma_a - \gamma_{pa}} \quad (4)$$

$$M_{pa} = \frac{F^h - M_b \gamma_b^h}{\gamma_{pa}} - M_a \quad (5)$$

$$E_{pa} = \frac{X^h - E_b \gamma_b^h}{\gamma_{pa}} - E_a \quad (6)$$

where,

M_b , M_a and M_{pa} are the molar concentrations of chlorophylls b and a and pheopigment a in the extract.

E_b , E_a and E_{pa} are terms used to predict the error in M_b , M_a and M_{pa} due to other pigment(s) X . These are added to the respective molar concentration.

F^u , F^h and F^e are the fluorescence of the extract unacidified (F^u), at time T after addition of HCl (F^h) and at equilibrium (F^e).

X^u , X^h and X^e are the fluorescence of other pigment(s) as described for F above.

γ represents the molar fluorescence coefficients (fluorescence/molarity) for the various pigments at excitation and emission peaks for chlorophyll a , as follows:

γ_a chlorophyll a

γ_{pa} pheophytin a

γ_b^u chlorophyll b

γ_{pb} pheophytin b

γ_b^h chlorophyll/pheophytin b mixture at time T after first HCl addition. This is a weighted average of γ_b^u and γ_{pb} , where the weights are the fractional molar proportions of chlorophyll b and pheophytin b at time T .

These equations are applicable to the kinetic method with three measurements of fluorescence and will fully eliminate chlorophyll b interference. For the alternative method with two fluorescence measurements (initial, F^u ; after HCl addition, F^h), the chlorophyll b terms (M_b , E_b) are set to 0 and equa-

tions (3) to (6) are used to calculate chlorophyll *a* and pheopigment *a* concentrations. In this modified form, equations (3) and (5) are identical to equations (1) and (2) of Holm-Hansen et al. (1965), where their *F* is the inverse of γ_a , and their maximum acid ratio 3.0 is γ_a/γ_{pa} . The accuracy of estimates of chlorophyll and pheopigment *a* made with two fluorescence measurements in the presence of chlorophyll *b* depends on the extent of chlorophyll *b* pheophytinization before the second measurement. As shown below, two measurements can give good accuracy for chlorophyll *a* if reaction time is optimal for the acid addition used.

Chlorophylls *a* and *b* purified by paper chromatography were used to develop and test the procedures. Pigments were extracted from grass (mainly *Dactylis glomerata* L) using absolute methanol and transferred to petroleum ether (b.p. 60 to 80 C) according to Loftus & Carpenter (1971). This extract was applied to Whatman 3MM paper, and the pigments were separated by ascending chromatography using petroleum ether + acetone (75:25 v:v). Chlorophylls *a* and *b* were eluted from the developed chromatograms with acetone (neutralized with $MgCO_3$), and these extracts, stored at -20 C, were used for reaction rate studies and for calibration purposes. The chlorophylls were transferred to diethyl ether for purity checks after evaporation of the acetone under N_2 . No impurities were detected photometrically or fluorometrically in the chlorophyll *a* preparation (Table 1). The chlorophyll *b* extract contained a slight chlorophyll *a* contamination which was considered in all calculations. Pigment concentrations in both extracts were determined

in 90% acetone according to Jeffrey & Humphrey (1975).

Conversion rate determinations for chlorophyll *c* were done with a fresh 90% acetone extract from *Fucus* sp., and fluorescence measurements were begun 0.5 min after acidification to allow reaction of most of the chlorophyll *a*. For determination of molar fluorescence coefficients, a crude chlorophyll *c* extract was prepared from *Fucus* sp. by the phase separation procedure of Strickland & Parsons (1968). Pigments were extracted from the acetone phase with diethyl ether and separated by paper chromatography according to Jeffrey (1961, chromatography in first direction only). The chlorophyll *c* ($c_1 + c_2$) plus some residual chlorophyll *a* was eluted in 100% acetone and calibrated according to Jeffrey & Humphrey (1975).

MgCO₃ suspension is commonly used in pigment analyses as a filtration aid and to provide basic conditions during extraction. To simulate this and still insure uniformity among samples, all 90% aqueous acetone solvent used was shaken in one batch with excess MgCO₃, which was then removed by filtration (Whatman GF/F). The resulting alkalinity in the solvent, 5.8 μ equiv., was insignificant compared to the acid concentrations used (1.0 to 4.0 m equiv.), however, and was ignored in the calculations.

Fluorescence was measured with an Aminco-Bowman spectrofluorometer equipped with an R-136 photomultiplier. The relationship between fluorescence and chlorophyll concentration was linear over at least the two most sensitive ranges of the instrument, where all measurements were made. Excitation and emission wavelengths for chlorophylls *a*, *b* and *c* were 430 and 670 nm, 460 and 654 nm, and 448 and 638 nm, respectively.

Bandwidths were 15 nm for routine measurements. Reaction rate constants were determined with fluorescence measured at the excitation and emission maxima for each chlorophyll, while determination of pigments in pure and crude extracts required measurements at chlorophyll *a* wavelengths only. An emission filter RG 610 (Schott & Gen., Mainz) was included to reduce stray light, and frequent checks of instrument stability were made using a uranium glass standard. The pheophytinization reaction is temperature dependent (Schanderl et al. 1962), so extracts were held in a 25 C water bath approx. 5 min before fluorescence measurements.

Chlorophylls *a*, *b* and *c* ($c_1 + c_2$) in extracts from natural phytoplankton were determined spectrophotometrically with the trichromatic equation of Jeffrey & Humphrey (1975). Chlorophyll *a* and pheopigment *a* were measured in the same extracts according to Lorenzen (1967), but with chlorophyll *a* absorptivity from Jeffrey & Humphrey (1975). Absorbance was measured with a Zeiss PMQ II spectrophotometer at 1.6 nm bandwidth.

The importance of the method of HCl addition to the cuvette was evident in preliminary tests of the kinetic method. Several different procedures were studied using dyed HCl solutions where the rapidity of mixing could be evaluated. The easiest and most rapid procedure was the following: after determination of initial fluorescence with 2.0 ml extract in the cuvette, 20 μ l HCl solution was expelled from a constriction pipette to the middle of the cuvette, and a stopwatch was started simultaneously. The tip of the pipette was immediately lowered to the bottom of the cuvette, and air was expelled until 5 sec had elapsed, thoroughly mixing the contents.

The pheophytinization reaction has been shown to be first order with respect to chlorophyll for chlorophylls *a* and *b* (Mackinney & Joslyn 1940; Schanderl et al. 1962). Chlorophyll concentrations in the extracts used for rate constant determinations were 4 to 7 nM. Since 1 to 4 mM HCl was added, $[H^+]$ was considered constant and conversion of chlorophyll to pheophytin was studied as a pseudo first order overall reaction. For determination of first order rate constants, HCl was added to the purified chlorophylls as described above to final concentration 1.0, 2.0, 3.0 or 4.0 mM. Fluorescence was measured manually for reaction times from 20 sec to at most 3 min. If necessary, conversion was then completed for measurement of equilibrium fluorescence by addition of 20 μ l 1.0 M HCl and further reaction until fluorescence was stable. Fluorescence was converted to percent undegraded chlorophyll, the natural logarithm of which was plotted against time. The slope of this line is the negative pseudo first order rate constant which is a function of the true rate constant and some power of $[H^+]$.

For all measurements with the kinetic method in natural extract, 20 μ l 0.3 M HCl was added to 2.0 ml sample (Final $[H^+]$ 3.0 mM). The second measurement of fluorescence was made after a reaction time of 52 sec, after which an additional 20 μ l 1.0 M HCl was added to the sample (total $[H^+]$ 13 mM). The third measurement was then made at equilibrium after an additional 90 sec. The four χ values were determined on each measurement occasion using the purified pigments, but the frequency of this calibration is determined by the stability of the fluorometer. Crude pigment extracts were diluted to a nominal 5 nM chlorophyll *a* for fluorometric measurements.

RESULTS AND DISCUSSION

The reaction curves used to determine rate constants showed good linearity, with most coefficient of determination values between 0.998 and 1.000 (Fig. 2). This showed the suitability of the HCl addition and mixing procedures and of the pseudo first order kinetics model. The mean intercept value for chlorophyll *a* curves was 100% (n 36, SD 2.1), but for chlorophyll *b* the mean intercept was significantly different from 100% ($\bar{x}$ 96%, SD 0.85, n 48, $p < 0.001$). This indicates the presence of some faster-reacting component, perhaps allomerized pigment as described by Schanderl et al. (1962). The portion of the curves ($t > 0.5$ min) used to determine chlorophyll *b* rate constants was not affected, however.

Mackinney & Joslyn (1940) concluded that the pheophytinization of chlorophylls *a* and *b* is a first order reaction with respect to H^+ . For the present data, however, regression of $\log_{10}$ of the pseudo first order rate constants (Table 2) on $\log_{10} [H^+]$ indicated a reaction order for H^+ of 2.0 (chlorophyll *a* 2.0, *b* 1.9; Fig. 5). Cho (1966) also found a second order reaction with respect to H^+ and suggested a reaction mechanism to explain the slower pheophytinization of chlorophyll *b*. Mackinney & Joslyn (1940) used oxalic acid in their reaction rate experiments. Dissociation of especially the second carboxyl group would probably be far from complete at the acid concentrations used. This would result in a lower apparent first order rate constant than when a strong acid is used at equivalent normality. In addition, lower

dissociation at higher concentrations may explain why Mackinney & Joslyn (1940) found first, instead of second, order kinetics with respect to H^+ .

The time necessary for 99% pheophytinization of chlorophyll *a* was calculated from the reaction rate constants (Table 2). Mean 99% conversion times were 47 and 29 sec for 3 and 4 mM HCl, respectively. Higher acid concentrations are unnecessary for pheophytinization in 90% acetone and can result in other, undesirable reactions (Riemann 1978; Moed & Hallegraeff 1978). The two parameters necessary to apply the kinetic methods were estimated from the rate constants (Table 2):

- 1) The time *T* for the second fluorescence measurement, which was taken as the mean 99% conversion time + 2SD.
- 2) The percent conversion of chlorophyll *b* at the time *T* of the second measurement.

Acidification to 3.0 mM H^+ was selected as giving a reaction rate for chlorophyll *a* large enough for rapid analysis but slow enough for convenient manual measurements. The corresponding *T* was 0.87 min (52 sec), giving weights for calculation of γ_b^h of 0.17 and 0.83 for γ_{pb} and γ_b^u , respectively (Table 2).

The three fluorometric methods were compared using a series of samples containing 5 nM purified chlorophyll *a* and varying concentrations of purified chlorophyll *b* (Fig.3). For the alternative with two fluorescence measurements, use of equilibrium fluorescence values after acidification resulted in large errors in calculated pigment concentrations, and these

increased with increasing chlorophyll *b* content. This was also shown by Loftus & Carpenter (1971) and will result from the use of equations like those of Holm-Hansen et al. (1965) if acid addition and reaction time permit significant conversion of chlorophyll *b* present in the extract. The ratio chlorophyll *b/a* in single algal species usually falls in the range 0.3 to 0.5 (Meeks 1974). In planktonic marine algae, for example, 27 species from 16 genera had a mean chlorophyll *b/a* ratio of ca. 0.5 (Wood 1979). At this value, acidification to equilibrium would result in underestimation of chlorophyll *a* by 23%.

In the second case, pigment values were calculated using two fluorescence measurements, but with the second measurement at optimal time *T* (52 sec) after acidification. These values were within 5% of theoretical concentrations up to an approximate chlorophyll *b/a* ratio 0.6 (Fig. 3). Pheopigment *a* was still grossly overestimated in the presence of chlorophyll *b*, however.

Three fluorescence measurements (F^u , F^h and F^e) allowed full correction for chlorophyll *b* and resulted in empirical chlorophyll *a* values within 5% of the theoretical level at all chlorophyll *b* concentrations tested (Fig. 3). In addition, the overestimation of pheopigment *a* was eliminated. Except for the sample with the lowest chlorophyll *b/a* ratio, the calculated concentration of chlorophyll *b* was also within 5% of the theoretical value (Fig. 4).

Extracts from *Fucus* sp. were used to test the effect of chlorophyll *c* ($c_1 + c_2$) on pigment determinations with the three fluorometric methods. Conversion to pheoporphyrin *c* was

also found to follow pseudo first order kinetics. The rate constant, 0.482 at 3.0 mM HCl, was intermediate between those of chlorophylls *a* and *b*. The chlorophyll *c* preparations faded somewhat (2 to 13%) during measurements, so the accuracy of the resulting fluorescence coefficients is uncertain. They were used, however, together with the rate constant to estimate chlorophyll *c* interference with eq. (2), (4) and (6) (Table 3). With the use of initial fluorescence plus that at equilibrium after acidification, 1.0 nM chlorophyll *c* is measured as 0.10 nM chlorophyll *a* and 0.15 nM pheopigment *a*. Use of two fluorescence measurements, but with fluorescence at optimal reaction time *T* instead of that at equilibrium, decreases chlorophyll *c* interference in determination of chlorophyll *a*, but increases error in pheopigment *a*. Addition of the third fluorescence measurement gives even lower interference in chlorophyll *a* but higher in pheopigment *a* (Table 3).

To test the fluorometric methods with natural phytoplankton samples, photometric and fluorometric determinations of chlorophylls and of pheopigment *a* were made on a series of extracts (fluorometric measurements after dilution) (Table 4). Since the fluorometer was calibrated with purified pigments, these determinations were completely independent. Extracts with no chlorophyll *b* showed no change in fluorescence after reaction time *T* (52 sec), and the three fluorometric methods performed identically. No effects of chlorophyll *c* were seen, since levels were not high enough in these samples to affect fluorescence after the 52 sec meas-

urement. However, even low concentrations of chlorophyll *b* gave differences between the equilibrium method and the two methods utilizing reaction time *T*. Differences in chlorophyll *a* between the two latter methods were only apparent at the highest chlorophyll *b* concentrations. Chlorophyll *b* effects on pheopigment estimates were apparent at all levels (Table 4).

Photometric (Lorenzen 1967) and the two fluorometric methods utilizing reaction time *T* gave similar results for chlorophyll *a*, but comparison is complicated by the fact that Lorenzen's method is also subject to some chlorophyll *b* interference. For the first 11 samples of Table 4, with little or no chlorophyll *b*, the difference between photometric chlorophyll *a* and the fluorometric value (method 2) ranged from -2.5 to +8.0% of the photometric value. The mean difference was +3.0%, which might represent a slight positive bias for the fluorometric determination. Comparison between photometric and fluorometric pheopigment *a* showed differences of from +3.2 to +80% ($\bar{x}$ +36%) for the 7 samples with little chlorophyll *b* and with photometric pheopigment *a*/chlorophyll *a* ratios of at least 0.1.

The effect of including a non-equilibrium fluorescence measurement (at time *T*) on the precision of chlorophyll *a* estimates was evaluated by determining the chlorophyll *a* concentration in 5 replicates of two pigment mixtures. The first, which contained 4.00 nM chlorophyll *a* and 1.00 nM pheophytin *a*, was measured before acidification and at equilibrium. The second mixture contained an additional 2.00 nM chlorophyll *b* (chloro-

phyll b/a 0.5), and measurement of fluorescence after acidification was made at 52 sec. Coefficients of variation for calculated chlorophyll a were 1.0 and 1.5% for the two series, respectively, showing little effect of the modified procedures on analytical precision.

Loftus & Carpenter (1971) tested the effects of various carotenoids on chlorophyll a fluorescence before and after acidification and found that, although absolute fluorescence values varied, the change upon acidification was constant. Measurement of chlorophyll a would not be affected, although pheopigment a might not be accurately estimated. In general, determination of pheopigment a is subject to more interference than is determination of chlorophyll a , since the calculation is based on a single measurement of fluorescence rather than the difference between initial fluorescence and that after acidification. Any constant red fluorescence, for example from pheopigment b , will result in positive pheopigment a error. While correction of chlorophyll a fluorescence for that of apparent pheopigment is certainly warranted, the non-specificity of pheopigment a estimation limits the use of the resulting values as such. This conclusion is supported by the poor agreement between photometric and fluorometric pheopigment a measurements shown above.

The spectrofluorometer used in this work allowed free selection of wavelength and bandwidth for excitation and emission light. The improved specificity for chlorophyll a demonstrated for the two kinetic methods should be of even greater use with a filter instrument where wavelength se-

lection is more difficult. One difficulty in using a spectrofluorometer arises from the extreme sensitivity of pheophytin *a* fluorescence to changes in excitation wavelength. This was probably due to the steeply-sloping absorbance spectrum around 430 nm and resulted in ca. 10% change in emission intensity with a nominal 1 nm shift in excitation wavelength.

In the present work 90% acetone was used as extraction solvent. Methanol is a more efficient solvent, however, especially for Chlorophyta (Marker 1972, Riemann in press). Optimal reaction time is probably different, but the general principle of a kinetic separation of chlorophylls *a* and *b* might also be useful in methanol. Moed & Hallegraeff (1978) found that formation of pheophytin *a* dications began at higher apparent pH in methanol than in acetone solutions. While Holm-Hansen & Riemann (1978) found no effect of this on fluorescence, further investigation of this and of reaction kinetics are necessary before the techniques shown here are applied to other solvents.

ACKNOWLEDGEMENTS

Dr. B. Riemann and Dr. A. Södergren made many constructive comments on the manuscript. Prof. L.O. Björn kindly advised me concerning the fluorometric analyses.

REFERENCES

- Abaychi, J.K., and J.P. Riley. 1979. The determination of phytoplankton pigments by high-performance liquid chromatography. *Anal. Chim. Acta* 107:1-11.
- Cho, D.H. 1966. Kinetics of the acid catalyzed pheophytinization of chlorophylls. Ph. D. Diss. Univ. of California, Davis. USA. 86 p.
- French, C.S. 1960. The chlorophylls *in vivo* and *in vitro*, p. 252-297. In W. Ruhland (ed.), *Encyclopedia of plant physiology*, v. 5/1. Springer-Verlag.
- Holm-Hansen, O., C.J. Lorenzen, R.W. Holmes, and J.D.H. Strickland. 1965. Fluorometric determination of chlorophyll. *J. Cons. perm. int. Explor. Mer* 30:3-15.
- Holm-Hansen, O., and B. Reimann. 1978. Chlorophyll a determination: improvements in methodology. *Oikos* 30:438-447.
- Jeffrey, S.W. 1972. Preparation and some properties of crystalline chlorophyll c_1 and c_2 from marine algae. *Biochim. Biophys. Acta* 279:15-33.
- Jeffrey, S.W. 1961. Paper- chromatographic separation of chlorophylls and carotenoids from marine algae. *Biochem. J.* 80:336-342.
- Jeffrey, S.W., and G.F. Humphrey. 1975. New spectrophotometric equations for determining chlorophylls a , b , c_1 and c_2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanzen* 167:191-194.
- Loftus, M.E., and J.H. Carpenter. 1971. A fluorometric method for determining chlorophylls a , b , and c . *J. Mar. Res.*

- 29:319-338.
- Lorenzen, C.J. 1967. Determination of chlorophyll and pheopigments: spectrophotometric equations. *Limnol. Oceanogr.* 12:343-346.
- Mackinney, G., and M.A. Joslyn. 1940. The conversion of chlorophyll to pheophytin. *J. Am. Chem. Soc.* 62:231-232.
- Marker, A.F.H. 1972. The use of acetone and methanol in the estimation of chlorophyll in the presence of phaeophytin. *Freshwat. Biol.* 2:361-385.
- Meeks, J.C. 1974. Chlorophylls, p. 161-175. In W.D.P. Stewart (ed.), *Algal physiology and biochemistry*. Blackwell.
- Moed, J.R., and G.M. Hallegraeff. 1978. Some problems in the estimation of chlorophyll- *a* and phaeopigments from pre- and post-acidification spectrophotometric measurements. *Int. Revue ges. Hydrobiol.* 63:787-800.
- Riemann, B. In press. A note on the use of methanol as an extraction solvent for chlorophyll *a* determination: improvements in methodology. *Ergebn. Limnol.*
- Riemann, B. 1978. Carotenoid interference in the spectrophotometric determination of chlorophyll degradation products from natural populations of phytoplankton. *Limnol. Oceanogr.* 23:853-860.
- Schanderl, S.H., C.O. Chichester, and G.L. Marsh. 1962. Degradation of chlorophyll and several derivatives in acid solution. *J. Org. Chem.* 27:3865-3868.
- Strickland, J.D.H., and T.R. Parsons. 1968. A practical handbook of seawater analysis. *Fish. Res. Bd. Can. Bull.* 167.
- Whitney, D.E., and W.M. Darley. 1979. A method for the de-

termination of chlorophyll a in samples containing degradation products. Limnol. Oceanogr. 24:183-186.

Wood, A.M. 1979. Chlorophyll $a:b$ ratios in marine planktonic algae. J. Phycol. 15:330-332.

Table 1. Photometric and fluorometric tests for purity of the chlorophyll *a* and *b* preparations.

Pigment	Characteristic	Literature value	Reference
chlorophyll <i>a</i>	Abs. peaks diethyl ether	661-662 nm 428-429	French (1960)
	blue/red Abs. peak ratio diethyl ether	1.31	French (1960)
	red fluor. peak acetone	668 nm (90% acetone)	Jeffrey (1972)
	acid ratio ¹ 90% acetone	1.71	Loftus & Carpenter (1971)
chlorophyll <i>b</i>	Abs. peaks diethyl ether	643-644 nm 452-453	French (1960)
	blue/red Abs. peak ratio diethyl ether	2.91	French (1960)
	red fluor. peak acetone	651 nm (90% acetone)	Jeffrey (1972)
	acid ratio ¹ 90% acetone	1.54 1.49	Loftus & Carpenter (1971)

1. Ratio of Abs. at red peak before acidification to Abs. at red peak after.

Table 2. Pseudo first order rate constants at 25 C for pheophytinization of chlorophylls *a* and *b* in 90% acetone and different HCl concentrations. Corresponding values for T (the upper 95% CL for chlorophyll *a* 99% conversion time) and for percent chlorophyll *b* remaining after reaction time T are also shown. Chl *a*, n=18; chl *b*, n=16.

Pigment	[HCl] mM	Rate constant min ⁻¹ , $\bar{x}$ (SD)	99% conversion time for chl <i>a</i> min, $\bar{x}$ (SD)	T min	Percent chl <i>b</i> at time T %, $\bar{x}$ (SD)
chlorophyll <i>a</i>	4.0	9.62 (0.34)	0.48 (0.017)	0.52	-
	3.0	5.81 (0.30)	0.79 (0.038)	0.87	-
	2.0	2.61 (0.13)	1.77 (0.082)	1.94	-
chlorophyll <i>b</i>	4.0	0.361 (0.026)	-	-	83 (1.2)
	3.0	0.220 (0.012)	-	-	83 (0.89)
	2.0	0.113 (0.005)	-	-	80 (0.79)

-: not applicable

Table 3. Chlorophyll *c* interference in fluorometric determination of chlorophylls *a* and *b* and pheopigment *a*. Errors are shown for the standard fluorometric method (1) and the alternative kinetic methods (2,3).

<u>Fluorometric method</u>	<u>Chl <i>c</i> at 1.0 nM is measured as:</u>		
	<u>chl <i>a</i></u> <u>nM</u>	<u>ph <i>a</i></u> <u>nM</u>	<u>chl <i>b</i></u> <u>nM</u>
1) 2 measurements (init., equil.)	+0.10	+0.15	-
2) 2 measurements (init., time T)	+0.03	+0.38	-
3) 3 measurements (init., time T, equil.)	+0.02	+0.50	-0.16

-: not applicable

Table 4. Photometric and fluorometric determination of chlorophylls *a*, *b* and *c* and pheopigment *a* (ph *a*) in extracts from natural phytoplankton. All values are in nM at the dilution used for fluorometric analyses. Photometric determinations were made with a trichromatic equation and according to Lorenzen (1967). Fluorometric determinations were made using initial fluorescence and fluorescence (1) at equilibrium after acidification; (2) at time T after acidification; and (3) at both time T and equilibrium after acidification. Samples are in order of increasing molar ratio *b/a*.

Sample	Photometry nM			Fluorometry nM			
	Trichromatic			Loren.	(1)	(2)	(3)
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i> (ph <i>a</i>)	<i>a</i> (ph <i>a</i>)	<i>a</i> (ph <i>a</i>)	<i>a</i> (ph <i>a</i>)
1.	4.99	0	0.89	4.71 (0.34)	-	4.86 (0.53)	-
2.	4.97	0	1.00	4.85 (0.10)	-	5.11 (0.09)	-
3.	4.83	0	0.91	4.74 (0.07)	-	4.99 (-0.20)	-
4.	4.99	0	1.07	4.97 (0.00)	-	5.03 (0.43)	-
5.	5.03	0	0.79	4.25 (1.25)	-	4.15 (1.29)	-
6.	4.97	0	0.70	4.57 (0.67)	-	4.73 (0.89)	-
7.	4.86	0	0.60	4.35 (0.92)	-	4.70 (0.96)	-
8.	4.92	0.06	0.64	4.53 (0.66)	4.65 (1.59)	4.71 (1.34)	4.73 (1.19)
9.	4.93	0.20	0.45	4.44 (0.82)	4.59 (1.51)	4.69 (1.13)	4.72 (0.90)
10.	5.00	0.28	0.40	4.57 (0.70)	4.45 (1.63)	4.56 (1.26)	4.58 (1.04)
11.	4.91	0.44	0.53	4.57 (0.55)	4.24 (2.71)	4.52 (1.65)	4.59 (0.97)
12.	4.70	1.28	0.09	4.29 (0.75)	3.98 (3.68)	4.58 (1.40)	4.74 (0.01)
13.	5.02	1.40	0	4.57 (0.78)	4.19 (3.31)	4.81 (1.15)	4.96 (-0.10)
14.	4.78	1.36	0	4.30 (0.86)	4.05 (3.44)	4.68 (1.23)	4.84 (-0.06)

-: No change in fluorescence after 52 sec; the fluorometric methods are equivalent.

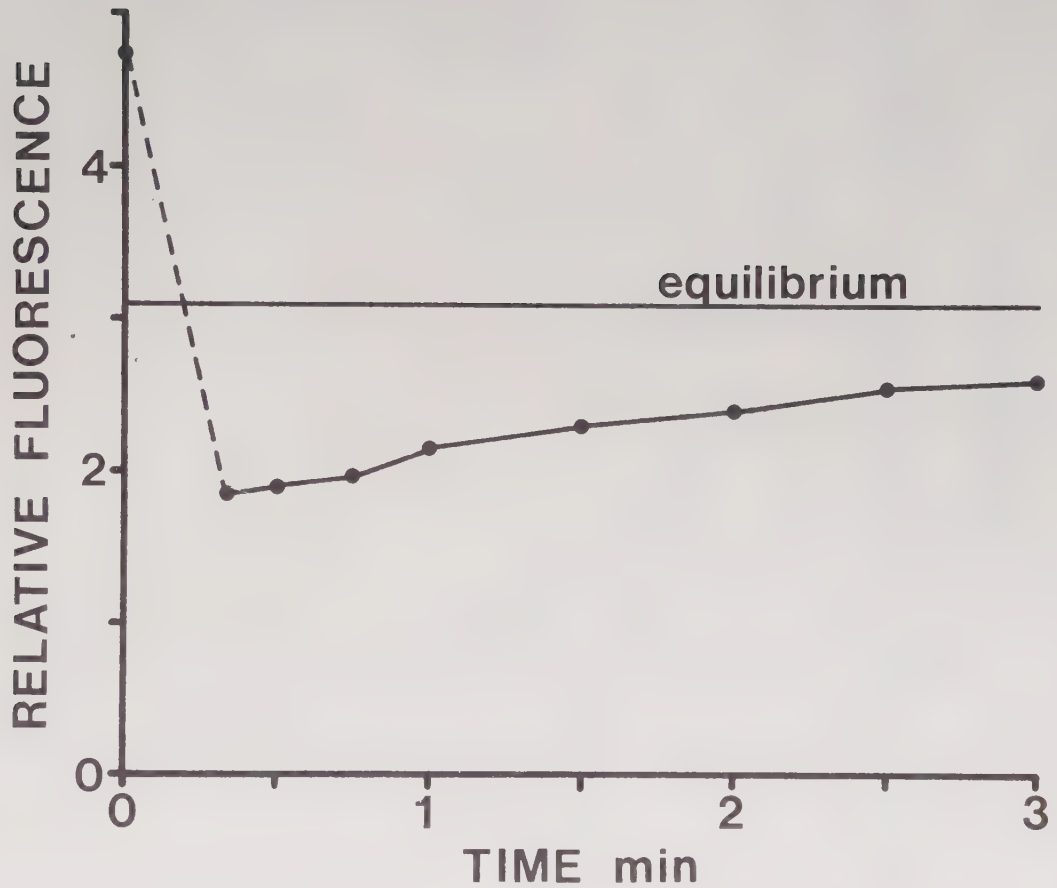


Figure 1. Fluorescence at chlorophyll *a* excitation and emission maxima as a function of time after acidification of a chlorophyll *a* + *b* mixture (molar ratio b/a 1.0). HCl was added to 4 mM at time 0. Solid line indicates equilibrium fluorescence after conversion of all chlorophyll *b*. No measurements were made between 0 and 0.33 min (dashed line).

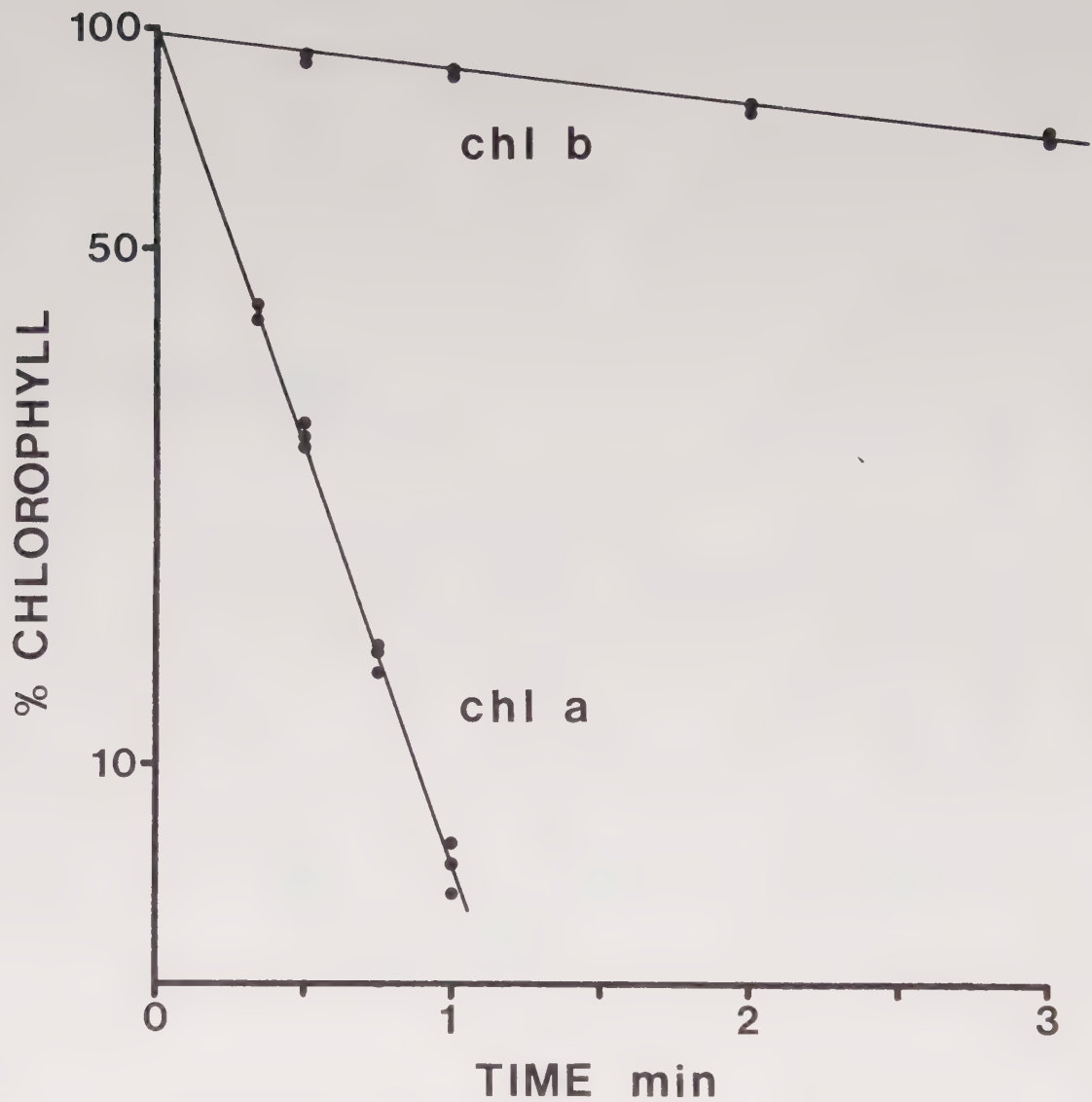


Figure 2. Example of reaction curves for pheophytinization of purified chlorophylls *a* and *b* at 25 C in 90% acetone. HCl (to 2 mM) was added to the extracts at time 0, and remaining chlorophyll (log scale) was measured fluorometrically with time. Lines show mean values (chl *a*, $n=18$; chl *b*, $n=16$), while points show three determinations chosen at random for each chlorophyll.

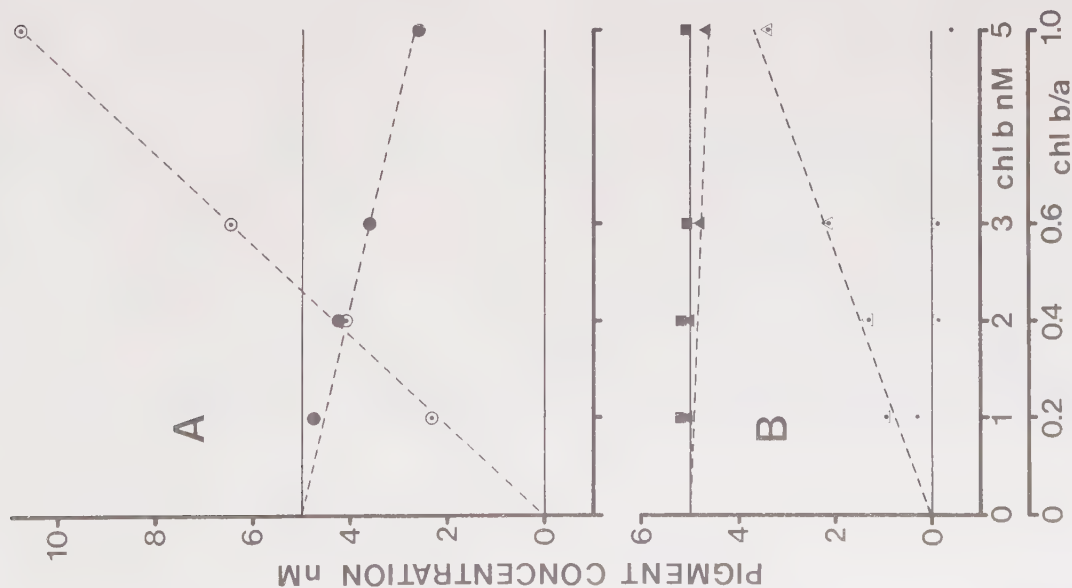


Figure 3. Effects of chlorophyll *b* concentration on estimates of chlorophyll and pheopigment *a* made with the different fluorometric methods. Samples contained 5.0 nM chlorophyll *a* and no pheopigment *a* (horizontal lines). Chlorophyll *b* was varied from 0 to 5.0 nM. Dashed lines show predicted values (eq. (2), (4), (6)), while points are actual measurements.

(A) Standard method using initial fluorescence and equilibrium fluorescence after acidification. Calculated chlorophyll *a* (●) and pheopigment *a* (○).

(B) Two variations of kinetic method. (▲, △) chlorophyll *a* and pheopigment *a*, respectively, calculated using initial fluorescence and fluorescence at time *T* after acidification. (■, □) chlorophyll and pheopigment *a* calculated with values for initial fluorescence and for fluorescence at time *T* and at equilibrium after acidification.

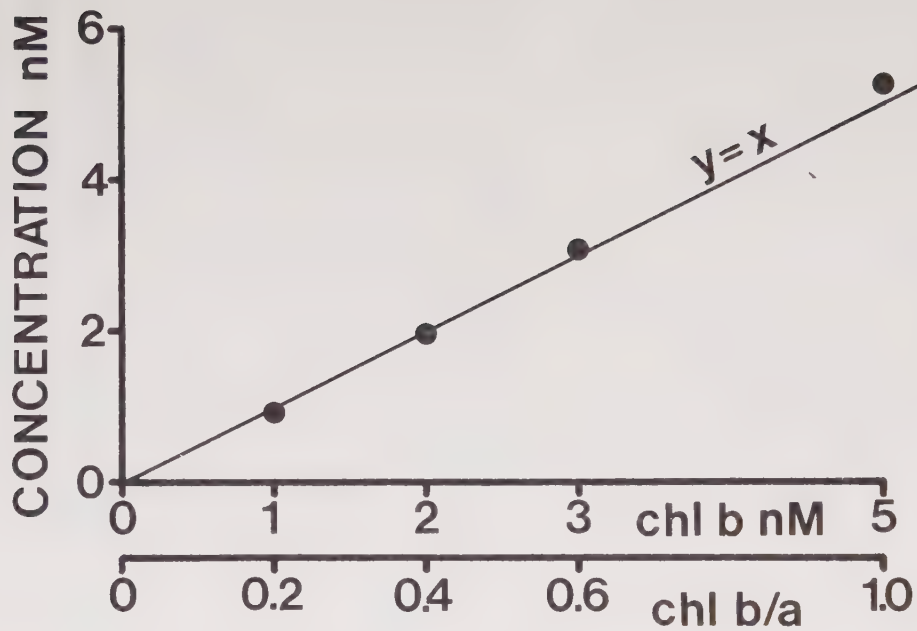


Figure 4. Chlorophyll *b* in mixtures containing 5 nM chlorophyll *a*, determined at chlorophyll *a* wavelengths using fluorescence at time *T* and equilibrium (eq.(1)). Chlorophyll *b* concentrations were 1, 2, 3 and 5 nM. Points show empirical values while line shows theoretical 1:1 relationship.

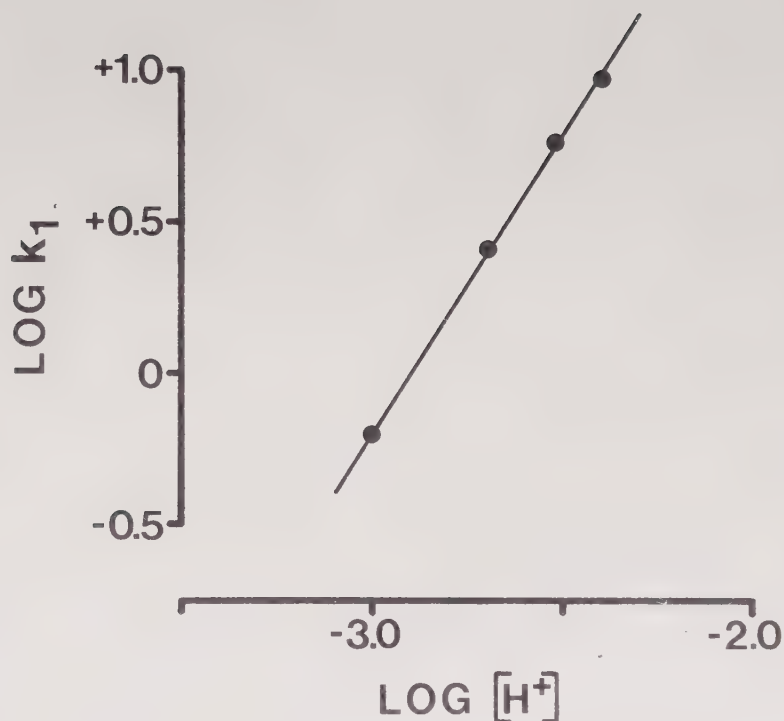


Figure 5. $\log_{10}$ of pseudo first order rate constant (k_1) for pheophytinization of chlorophyll *a* at 25 C as a function of $\log_{10} [\text{H}^+]$. Least-squares fit of log-log relationship: $k_1 = (5.21 \times 10^5) [\text{H}^+]^{2.0}$. Similar treatment of first order rate constants for chlorophyll *b* gave regression equation: $k_1 = (1.49 \times 10^4) [\text{H}^+]^{1.9}$. For each chlorophyll, rate constants from Table 2 were used together with a fourth value, determined at 1 mM H^+ for chlorophyll *a* and 10 mM for chlorophyll *b*.

Transport of Photosynthetic Carbon from
Phytoplankton to Bacteria in Freshwater
Plankton Communities

Michael F. Coveney

Institute of Limnology
Box 3060
S-220 03 Lund, Sweden



1980

CONTENTS

	<u>Page</u>
Abstract	1
Introduction	3
Methods and Materials	5
H^{14}CO_3 Uptake Experiments	5
Analyses	9
Calculations	11
Results	13
Discussion	17
Interpretation of Size Fractionation Data	17
<i>In Situ</i> Incubations	21
Time-Series Incubations	25
Microheterotrophic Uptake of Algal Extracellular Products	30
Acknowledgements	35
References	36
Tables	42
Figures	47

ABSTRACT

Movement of photosynthetic carbon from phytoplankton to dissolved material and small particles (bacteria) was studied in two eutrophic lakes in southern Sweden. Particulate and dissolved fractions were separated by membrane filtration (0.2 μm); initial fractionation through a Whatman GF/C filter was used to isolate small particles. Carbon (H^{14}CO_3) uptake in the three size fractions was determined in depth profiles *in situ*, and uptake kinetics were investigated in the laboratory. 60 to 80% of the bacteria passed the fractionating filter (GF/C) together with <1% of the chlorophyll a . ^{14}C fixed in algal photosynthesis was recovered in dissolved and small particulate fractions, and the small particulate ^{14}C probably represented bacterial uptake of excreted algal products. *In situ* extracellular release (dissolved + small particulate) was maximal at or near the surface. Release as a percent of total assimilation was usually minimal at the depth of maximal productivity, however. Despite inclusion of the small particulate fraction, percent release was low in these eutrophic systems, 1.2 to 6.8% of total ^{14}C uptake per m^2 lake surface. From 36 to 73% of the ^{14}C released was recovered in the small particulate fraction after ca. 4 h incubation. The carbon uptake rate to small particles *in situ* showed little variation in the different samples, while ^{14}C accumulation in the dissolved fraction varied inversely with water temperature. Addition of a mixture of D-glucose, glycine and sodium glycolate to samples before *in situ* in-

cubation did not prevent transport of ^{14}C to small particles, which implicated other than these substances in the release and uptake observed. Laboratory time-series incubations indicated steady state for the pool of algal extracellular products on one occasion, while increasing pool size was indicated in the remaining two experiments. For the experiment where steady state was demonstrated, percent gross release based on kinetics of dissolved ^{14}C (0.78%) was similar to the 0.60 to 0.80% calculated as dissolved + small particulate ^{14}C . Transport of photosynthetic carbon to small particles *in situ* was 32 to 95% of estimated heterotrophic bacterial production (as dark $^{14}\text{CO}_2$ uptake) on four occasions. Thus, while excretion was apparently not an important loss of carbon for phytoplankton in these samples, it may have represented an important carbon source for planktonic bacteria.

INTRODUCTION

The release of dissolved organic carbon from photosynthesizing algae has been intensively studied in recent years (for reviews, see Fogg, 1971; Hellebust, 1974; Nalewajko, 1977). Values for extracellular release in the range 5 to 30% of total carbon fixation are common, and up to 95% release has been found (Fogg, 1971). In a critical review of earlier work, Sharp (1977) described possible sources of error in the measurement of algal excretion and concluded that the range 0 to 5% of total carbon fixation is more realistic for marine phytoplankton.

The extreme variation in reported values for algal extracellular release probably reflects both differences in the accuracy of measurements and the wide variation in communities studied and experimental conditions. In addition, two processes affecting values for extracellular release obtained in ^{14}C tracer experiments have rarely been considered quantitatively: heterotrophic uptake of released, labeled compounds and the rate of algal intracellular turnover of the released material.

Heterotrophic uptake results in a direct loss of dissolved organic products formed during incubation and a resulting underestimation of extracellular release. Based on laboratory experiments, Sharp (1977) concluded that bacterial utilization does not mask an otherwise high excretion by marine phytoplankton. Other workers have found significant bacterial uptake of algal products in natural communi-

ties, however, using methods including size fractionation (Derenbach & Williams, 1974; Larsson & Hagström, 1979), inhibition of heterotrophs with antibiotics (Chróst, 1978) and compartmental analysis of the algae-dissolved organics-heterotrophs system (Wiebe & Smith, 1977a).

The turnover rate of algal intracellular pools of the material to be released determines how rapidly ^{14}C labelling in the excreted material rises to equilibrium with that of the inorganic carbon in the medium. The implicit assumption in most work is that turnover is rapid on the time scale of the experiment. If this is not the case, carbon is released that was fixed before tracer addition, ^{14}C is not a quantitative tracer for total carbon and release is underestimated. This problem has been discussed (Berman, 1975; Storch & Saunders, 1978), but very little quantitative work exists.

In the work presented here, size fractionation was used in ^{14}C uptake experiments to estimate microheterotrophic uptake of algal extracellular products in natural freshwater plankton communities. This transport of carbon from photosynthesizing algae to small particles (i.e. bacteria) was investigated both in depth profiles *in situ* and in time-series incubations in the laboratory. In this paper "excretion" is used synonymously with "extracellular release" to mean the liberation of dissolved substances from intact, living cells (Fogg, 1971).

METHODS AND MATERIALS

H¹⁴CO₃ Uptake Experiments

Experiments were done with the plankton communities in two small eutrophic hard-water lakes in southern Sweden, Lake Bysjön and Lake Häljasjön (general description of Lake Bysjön in Coveney et al., 1977). A PVC pipe was used to sample the layer 0 to 2 m in the pelagic area. This sample was mixed gently and used for all determinations. The experiments were conducted during March-April and September-October, 1978 (Table 1). The lakes were isothermal during both periods. The three spring and first fall experiments consisted of *in situ* incubations only. The final six experiments consisted of an incubation *in situ* one day followed by a laboratory incubation at the same temperature the following day (Table 1).

In situ incubations were based on standard ¹⁴C productivity methodology (Steemann Nielsen, 1952). The 0 to 2 m integrated sample was exposed at different depths for 4 h (11:00 to 15:00) in 50 ml borosilicate glass bottles. Bottles were suspended horizontally and contained 260 to 590 kBq (7 to 16 µCi) NaH¹⁴CO₃. One dark bottle was included in each profile and, during the fall series, a formalin-fixed blank (to 4% v:v) was also incubated.

Bottles were kept in the dark at *in situ* temperature until filtration, which was begun 15 to 30 min after incubation was terminated and required 1.0 to 1.5 h. Filtration

was by vacuum (max. pressure differential 49 kPa) through 25 mm diam filters in Millipore Swinnex holders. The modified syringes and procedure used in vacuum filtration have been described previously (Coveney, 1978).

Two 20 ml samples were removed by syringe from the well-mixed contents of each bottle (Fig. 1). The first was filtered through a pre-wetted 0.2 μm Sartorius membrane filter. The final 10 ml of this filtrate was collected, fixed with toluene and processed within 6 h (see below). The second sample was passed through a Whatman GF/C glass fiber filter (Coveney, 1978), and remaining particles were retained on a pre-wetted 0.2 μm Sartorius edge-hydrophobic membrane filter. All filters were rinsed with 10 ml 0.2 μm -filtered lake water. Filters were held in formalin and chloroform vapor during filtration of the remaining samples, fumed 10 min above concentrated HCl and placed in liquid scintillation vials after 10 min in air. The capped vials were then stored at -20 C until further treatment. This filtration procedure resulted in three size fractions for ^{14}C determination: total particulate ($> 0.2 \mu\text{m}$), dissolved ($< 0.2 \mu\text{m}$) and particulate passing a GF/C filter (Fig. 1).

Retention of dissolved organic ^{14}C on the 0.2 μm edge-hydrophobic filter would result in overestimation of particulate ^{14}C passing the GF/C filter. To quantify this, *in situ* samples were first filtered through a 0.2 μm membrane filter. The filtrate was refiltered and rinsed through the GF/C + 0.2 μm filter combination, and the 0.2 μm filter was saved for ^{14}C determination. The ^{14}C retained was compared

both to total organic ^{14}C in the first filtrate and to the ^{14}C which passed a GF/C filter but was retained on a $0.2\ \mu\text{m}$ filter in the first filtration.

In connection with the fall series of *in situ* experiments, the effects of two treatments on uptake and distribution of ^{14}C during incubation were investigated. First, samples were incubated with H^{14}CO_3 after removal of large particles by pre-fractionation. Portions of 100 ml water were passed under slight pressure through 47 mm diam GF/C filters, giving the same flow and only slightly (35%) greater filter loading than standard fractionation procedure. Light and dark bottles with pre-fractionated water and a non-fractionated control were incubated at 0.5 m in parallel with, but longer than the regular profile. Dissolved ^{14}C and small particulate ^{14}C in pre-fractionated samples were compared to the same fractions in control samples which were fractionated after incubation (post-fractionation).

In the second treatment, a mixed organic solution was added at 2 different levels to 2 bottles in an attempt to prevent heterotrophic utilization of labeled algal products by dilution with non-labeled organics. 1 and 5 mg C l^{-1} of D-glucose, glycine and sodium glycolate were added to the bottles, which were incubated at 0.1 m and filtered together with the regular profile.

Water for laboratory incubations was taken the morning of the experiment and held in dim light and *in situ* temperature. The incubations were done in single light (560 ml) and dark (125 ml) borosilicate glass bottles. Temperature was

regulated ± 0.2 C by a circulating water bath, with mean temperature within 0.5 C of that *in situ*. A quantum flux of ca. $150 \mu \text{ einsteins} \cdot \text{m}^{-2} \cdot \text{sec}^{-1}$ (400 to 700 nm) was supplied by fluorescent lights. Incubation was started by addition of H^{14}CO_3 to the bottles after a short (15 to 60 min) period under the experimental conditions. Two 20 ml subsamples were removed from the bottles at intervals over a ca. 5.5 h period and immediately filtered as described for field incubations. At about 2 and 5 h after start of incubation, 1 ml was removed from the light incubation bottle and diluted 100X with 0.05 M NaOH. ^{14}C activity was measured in a 1 ml aliquot of the diluted sample to check for loss of inorganic ^{14}C through exchange with the atmosphere. The effects of dark storage of samples before filtration were evaluated during the laboratory experiments by the transfer of a portion of the light sample to a dark bottle after 3 h incubation. Subsamples were removed after 1 and 2 h in the dark and filtered as above.

Retention of bacteria and algae on the fractionating filter (GF/C) was checked for each experiment. 20 ml samples followed by 10 ml rinse were drawn by vacuum through a GF/C filter, and the filtrate was retained for analysis. A thin cannula (0.80 mm OD X 50 mm) on the outlet of the Swinnex holder gave approximately the same resistance to water flow as the second, membrane filter in the true fractionations. Fractionated and whole water samples were fixed with Lugol's solution (Saraceni & Ruggiu, 1974) for phytoplankton examination and with glutaraldehyde (to ca. 5% v:v) for bacterial

counts. Samples for chlorophyll *a* were stored dark and cool for at most 8 h until filtration.

Analyses

^{14}C in particulate and dissolved fractions was measured with an Interteknique SL-31 liquid scintillation counter. Membrane filters were digested in scintillation vials ca. 5 h at 45 C with 1.0 ml Soluene 100 or Soluene 350 (Packard Inst. Co. Inc.). The resulting brown coloring was bleached by addition of 200 μl isopropanol, 200 μl 30% H_2O_2 and warming at 45 C ca. 20 min (Anon., 1974). 15 ml scintillation solution (4.7 g PPO, 130 mg POPOP, 667 ml toluene p.a., 333 ml 2-methoxyethanol p.a.) was added to each vial, and the contents were mixed. Counting was begun first after a 48 h delay for chemoluminescence decay. Quench correction was done by external standard channels ratio using a quenched series prepared with blank membrane filters digested as described. ^{14}C was added as hexadecane standard (Radiochemical Centre, Amersham, England), and acetone was used as quenching agent. The curve was checked frequently by internal standard.

10 ml samples for dissolved organic ^{14}C determination were acidified with 0.1 ml 1 M HCl and bubbled vigorously with air in test tubes for 30 min. The efficiency of inorganic ^{14}C removal using this procedure was tested for each batch of H^{14}CO_3 used. The slight residual activity (ca. $10^{-4}\%$) was subtracted from all experimental samples. After 30 min bubbling the tubes were capped and shaken well, and 8.00 ml

was removed and added to 8.00 ml Insta-gel (Packard Inst. Co. Inc.) in a scintillation vial. The vials were warmed to 40 C, shaken and allowed to cool undisturbed to counting temperature (12 C). Counting efficiency was determined by internal standard using a calibrated ^{14}C -glucose solution. Samples for total ^{14}C taken during laboratory time-series were counted by mixing 1 ml of the diluted sample with 9 ml Insta-gel. Counting efficiency was determined by internal standard using ^{14}C -hexadecane. For all samples, counting time was up to 120 min for low ^{14}C levels.

Chlorophyll *a* in whole samples was determined according to Lorenzen (1967) after grinding and 24 h extraction with 90% aqueous acetone. Fractionated samples were prepared and analyzed spectrofluorometrically as described previously (Coveney, 1978), except that a different modification of the method of Holm-Hansen et al. (1965) was used to eliminate interference by chlorophyll *b* (Coveney, in prep.). Frequent blank samples (0.2 μm -filtered lake water) and internal standards (1 ml whole lake water) were included in the fluorometric analyses.

Bacteria were counted by epifluorescence microscopy on 0.2 μm Nuclepore filters (Hobbie, Daley & Jasper, 1977). Cells were stained for 2 min in 0.008% acridine orange and filtered. The filter was air dried, mounted in immersion oil and examined at 1250X using a Zeiss Universal microscope. Illumination was provided by a HBO 200 Hg lamp, primary filtration was through BG 38, KP 500 and LP 450 filters and a FL-500 splitter, and secondary filtration was a barrier fil-

ter 53 (all Zeiss designations). About 400 cells were counted. Phytoplankton were examined by standard inverted microscope techniques.

Calculations

The fractionation procedure resulted in direct determination of ^{14}C in three size fractions: total particulate, dissolved and particulate passing a GF/C filter (Fig. 1). This third fraction included part of the particles in the size range of bacteria, while part was retained by the GF/C filter. Percent bacteria passing the GF/C filter in each experiment was used to correct for this retention of small particles. Thus, values for chlorophyll *a* and ^{14}C in particles in the GF/C filtrate were multiplied by the factor $100/A$, where *A* is percent bacteria in the filtrate, to obtain corresponding values for the small particulate fraction. ^{14}C in all fractions was converted to carbon according to Goldman, Steemann Nielsen, Vollenweider & Wetzel (1974), using total CO_2 determined from alkalinity and pH measurements (Rebsdorf, 1972) and the specific activity ($^{14}\text{C}/^{12}\text{C}$) of the dissolved inorganic carbon pool. As discussed above, this measures flux of ^{14}C -labeled material to the dissolved and small particulate pools, but may underestimate total carbon flux. Carbon uptake per m^2 lake surface was obtained by planimetry of vertical profiles of uptake into the different size fractions. In data treatment, carbon uptake into dissolved and small particulate fractions was expressed both in carbon units and as a percent of total uptake.

With the assumption that small particulate ^{14}C is derived from labeled algal products, the sum of dissolved and small particulate ^{14}C is a better estimate of true excretion than dissolved ^{14}C alone. Furthermore, the small particulate fraction can be expressed as a percent of this sum, and this is termed the percent transport to small particles.

Algal excretion data are found in the literature both as light values only and as light minus dark values. The choice depends on whether dark excretion is assumed to also occur in the light, or if it is felt to be a separate process (Fogg, 1971). Here, light values are used, but the corresponding dark values are included for comparison.

Since a single sample per depth or time was fractionated in the ^{14}C uptake experiments, the precision of the measurements was estimated separately. Water from Lakes Bysjön and Häljasjön was incubated in the laboratory and series of samples removed and fractionated. The resulting estimates for 95% CL of a single value were $\pm 8.8\%$ for both total particulate and dissolved ^{14}C and $\pm 13\%$ for small particulate ^{14}C . For occasional samples with exceptionally low ^{14}C levels, the uncertainty in ^{14}C determination exceeded these values and was used instead in significance tests.

RESULTS

H^{14}CO_3 uptake was low in all size fractions of formalin-fixed samples (Fig. 2; Fig. 3, formalin blanks). Removal of large particles by pre-fractionation greatly decreased ^{14}C uptake into dissolved and small particulate fractions in the light, while dark uptake into these fractions was unchanged or even increased (Fig. 2).

Light H^{14}CO_3 uptake in pre-fractionated samples was only 0.12 to 0.55% of that in the whole water sample (Table 2). Subtraction of dark uptake decreased the percentage even more. In contrast, small particulate ^{14}C in post-fractionated samples was a greater percent of the total (0.52 to 2.4%), and subtraction of dark values resulted in only slight decreases (Table 2).

Only a small fraction (0.10 to 1.2%) of total algal pigment fluorescence was found in the small particulate fraction (Table 2). The percentage chlorophyll *a* was even lower (0.042 to 0.86%), since the small particulate material had a high content of other fluorescent matter. These low chlorophyll *a* values were not due to pigment losses before analysis, since recovery of internal standards (1.0 ml whole lake water) with the fluorometric method was 85 to 107% ($\bar{x}$ 94%) of the value expected from photometric determinations on whole water. Blank samples (0.2 μm -filtered lake water) always contained some material measured as chlorophyll *a*, but these were not subtracted from sample values since the nature of this material was not determined.

These estimates of algal biomass in the small particulate fraction (H^{14}CO_3 uptake after pre-fractionation, chlorophyll α) were low compared to the 62 to 84% of bacteria passing the GF/C fractionation filter (Table 3). On three occasions, water was fractionated after 60 min dark incubation with D-(U^{14}C)glucose. More glucose uptake activity than bacterial cells was retained on the GF/C filter in two of the tests (Table 3).

The retention of dissolved ($\leq 0.2 \mu\text{m}$) organic ^{14}C on the $0.2 \mu\text{m}$ edge-hydrophobic membrane filter (used to collect small particulate ^{14}C , Fig. 1) was tested by passing a $0.2 \mu\text{m}$ filtrate through the GF/C + $0.2 \mu\text{m}$ filter combination. Except for a single anomalous value (32%), mean retention of organic ^{14}C from the first filtrate was a negligible 6.6% (Table 4). The single high value was probably due to improper seating of the membrane filter in its holder during the first filtration. This mean retention during refiltration was equivalent to 5.6% of the organic ^{14}C retained by the same filter in the first filtration, which indicates nearly quantitative removal of this material in one filtration step.

In the *in situ* experiments, dark fixation of H^{14}CO_3 was present in all size fractions, although values were small compared to light values in the upper photic zone (Fig. 3). Dark dissolved ^{14}C on 29 March was an exception, but this extreme value must have been a result of leakage during filtration. Light fixation into small particulate and dissolved fractions roughly followed total ^{14}C uptake, maximal at or just under the surface and minimal towards the bottom (Fig. 3). Expressed as percent of total uptake, however; values for

both the small particulate and dissolved fractions were generally minimal at the productivity maximum. They increased slightly towards the surface and markedly towards the bottom.

Expressed per unit area, dissolved ^{14}C represented 0.50 to 4.2% of total uptake, while small particulate ^{14}C was 0.75 to 2.6% on the same basis. The sum, an estimate of gross excretion during incubation, was at most 6.8% (3 April, Fig. 3). The percent transport to small particles varied only slightly within each vertical profile, with some exceptions. Percent transport was high, ranging on a unit area basis from 36% in spring to 73% in the fall (Fig. 3).

Total light ^{14}C uptake was linear during the ca. 5.5 h laboratory experiments (Fig. 4). Dark uptake was measured at ca. 2 and 5 h and was insignificant at those times in all fractions. Fixation into small particulate and dissolved fractions showed varying patterns - including both linear and asymptotic increases with time - and these were reflected in changes in the percent transport values (Fig. 4). Values for percent ^{14}C in the dissolved and small particulate fractions taken at 4 h in laboratory experiments were close to those measured one day earlier *in situ* (Fig. 3B). A check of total ^{14}C content in the water phase during the three time-series showed some losses to the atmosphere. Total ^{14}C in the incubation vessel decreased by a mean of 9% during 2 h and 12% during 5 h. These losses were ignored in interpreting the time-series results.

Addition of a dissolved organic mixture to samples had no consistent effect on distribution of ^{14}C during subsequent

incubation with H^{14}CO_3 (Fig. 5). ^{14}C accumulated in the small particulate fraction of all samples despite the large addition of unlabeled organics. Decreased small particulate ^{14}C and an increase in the dissolved fraction occurred on only two of the occasions (7 September, 11 October). In one experiment (11 October) the higher organic addition significantly stimulated total ^{14}C uptake.

DISCUSSION

Interpretation of Size Fractionation Data

Comparision of formalin-fixed, pre-fractionated and post-fractionated samples indicated that the ^{14}C accumulated in both the dissolved and small particulate fractions was assimilated in algal photosynthesis (Fig. 2). Little ^{14}C was found in these fractions in fixed samples, which showed that residual H^{14}CO_3 was effectively removed. This can otherwise give serious errors with both filtrate (Sharp, 1977) and filter (Lean & Burnison, 1979) samples. Furthermore, both dark incubation and pre-fractionation (i.e. removal of algae) resulted in greatly lowered ^{14}C activity in both fractions (Fig. 2). Direct $^{14}\text{CO}_2$ uptake by microheterotrophs was therefore not quantitatively important.

Damage to phytoplankton cells during filtration could give false, high values for ^{14}C in both the dissolved and small particulate fractions. Lean & Burnison (1979), in an examination of both earlier and new data, concluded that cell damage is rare unless large sample volumes are filtered. In addition, both dissolved and small particulate ^{14}C expressed as percent of total uptake varied predictably with depth *in situ* (Fig. 3), despite the fact that all bottles were filled from a single mixed water sample. This variation is not consistent with cell damage, since damage would have been the same at all depths.

Small particulate ^{14}C could have resulted from small auto-

trophic organisms passing the GF/C filter. However, ^{14}C accumulation in the small particulate fraction was much greater than the apparent contamination of that fraction by algae (Table 2). As a rule, small particulate chlorophyll *a* and H^{14}CO_3 uptake in pre-fractionated samples were lower percentages of whole water values than was small particulate ^{14}C with post-fractionation. The changes in percent small particulate ^{14}C observed with depth *in situ* (Fig. 3) would not be expected if this ^{14}C were due to small autotrophs, since the proportion of small cells would be identical at each depth. Furthermore, there was a relatively stable relationship between small particulate and dissolved ^{14}C at different depths in each *in situ* experiment, which is not expected if the two fractions have independent sources.

At least two processes could give the patterns for dissolved and small particulate ^{14}C observed here: 1) uptake by microheterotrophs of dissolved organic ^{14}C released from algae; and 2) abiotic formation of colloidal/particulate ^{14}C from labeled algal products (Nalewajko, 1977). S ndergaard (1980) found that macrophytes incubated with H^{14}CO_3 in flow-through chambers released organic ^{14}C , from 20 to 65% of which could be collected on 0.2 μm membrane filters. Since the inflowing water was bacteria free, he concluded that this particulate ^{14}C probably represented abiotic adsorption or aggregation of released organic matter. The possibility of sloughing-off of labeled epiphytic bacteria was not directly checked, however.

In the present work the small particulate organic ^{14}C was

almost quantitatively removed by one filtration; a second filtration gave only an additional 5.6% (mean value, Table 4). A similar result was found by S ndergaard (1980) using refiltration on two different membrane filter materials. Thus this material behaved as particles larger than ca. 0.2 μm , rather than labeled colloids which should have shown higher refiltration values. The small particulate material isolated by size fractionation in this study was rich in bacteria (Table 3), contained almost no algae and accumulated organic ^{14}C in the presence of algae and in the light. While the identity of this fraction is not completely clear, I conclude that it probably represents microheterotrophic (i.e. bacterial) uptake of labeled algal excretion products.

Direct counts of bacteria were used as a measure of retention of small particles on the fractionation filter, i.e. to calculate ^{14}C in the entire small particulate fraction from particulate ^{14}C in the GF/C filtrate. For the success of this procedure, bacterial cells and uptake activity for algal products must be distributed equivalently in the two fractions. Tests on three occasions using glucose uptake activity showed that the differences were not serious (Table 3). Similarly, Hobbie & Wright (1979) showed an example of good agreement between distribution of bacteria in five particle size classes and distribution of glutamate uptake activity in an estuarine sample.

In the absence of respiration of the small particulate ^{14}C , the sum of dissolved and small particulate ^{14}C is an estimate of the true (gross) excretion of labeled products during the experiment. This is true regardless of the kinet-

ics of release or of small particulate uptake. If the small particulate activity does represent microheterotrophic uptake, then respiration at some level is probable, and gross release will be underestimated if respiration is ignored. Wiebe & Smith (1977a) found only 2 to 4% respiration of assimilated algal excretion products in two estuarine samples, while Iturriaga & Hoppe (1977, calculated from Fig. 3) found 19, 20 and 31% in three samples from the Baltic Sea. Nalewajko (1977, calculated from Table 8.1) reported values in cultures ranging from 0 to 93% for four algal species and two bacterial strains. This wide variation makes choice of a respiration value impossible, so no respiration correction was applied to the data reported here.

Dissolved organic ^{14}C has been interpreted as a direct algal product in this and most other studies, but it is clear that bacteria also release organic carbon to the medium (Nalewajko & Lean, 1972). Dunstall & Nalewajko (1975) measured excretion of labeled products by *Pseudomonas fluorescens* during growth on glucose, fructose and sucrose. Extracellular release was minimal during the first 10 h of the experiments. In their tests, natural bacteria showed low release levels after 24 h incubation with glucose, glycolate and alanine. Thus while bacterial excretion certainly occurs, there is no evidence that release of labeled organic material from bacteria would have been important in the experiments discussed here.

In Situ Incubations

The *in situ* vertical pattern of percent extracellular release (sum of dissolved + small particulate ^{14}C) in the light was found to be that described for dissolved ^{14}C in earlier work (e.g. Watt, 1966), minimal at the level of maximal productivity and increasing at both lesser and greater depths (Fig. 3). Smith & Wiebe (1976) found constant extracellular release in natural phytoplankton communities and algal cultures despite a decrease in total carbon assimilation caused by artificial carbon limitation. They postulated that varying percent extracellular release is due to constant absolute release in connection with varying total ^{14}C assimilation. The increase in percent release observed under light-limited conditions *in situ* clearly did not depend on such a mechanism, however. In most profiles, absolute release varied directly with total assimilation from the depth of maximal productivity downwards (Fig. 3). The increase in percent release with depth persists, although to a lesser degree in some cases, if dark dissolved and small particulate ^{14}C values are subtracted from light. This leaves most of the percentage values at 2, 3 and 4 m unchanged (Fig. 6). Extracellular release does not appear to consist of a constant, light-independent (dark) part with high percent release plus a variable light-dependent part with low percent release. Rather, there is an apparent change in overall release characteristics with depth (light) in the water column.

On a square meter basis, extracellular release (dissolved

+ small particulate) in the light was from 1.2 to 6.8% of total productivity. From 36 to 73% of this was found in the small particulate fraction after ca. 4 h (Fig. 3), indicating the importance of short-term transport to small particles. Despite inclusion of the small particulate fraction, however, the percentage release values reported here are low compared to many reports in the literature (Fogg, 1971; Hellebust, 1974; Berman & Holm-Hansen, 1974). The low net excretion levels observed in eutrophic Lake Bysjön (Fig. 3, Coveney et al., 1977) apparently were not a result of bacterial activity. Instead, gross release was low, close to the 0 to 5% suggested by Sharp (1977) for marine phytoplankton.

The percent transport values show how much of the released, labeled carbon became rapidly associated with small particles. If the small particulate ^{14}C represents microheterotrophic uptake, then this transport can be expected to be a function both of the activity of the heterotrophs and of the suitability of the excreted material for heterotrophic utilization. Temperature varied at most 0.7 C at the depths of incubation within a single *in situ* profile. Heterotrophic activity should, therefore, have been similar at all depths, and the small variation in percent transport with depth indicates that the suitability of excreted material did not vary greatly. This is true despite the large variations in absolute and percent release.

Percent transport on a square meter basis increased almost linearly with temperature over the range 5 to 16 C (Fig. 7). A higher percentage of the released material was assimilated by microheterotrophs at higher temperature, and

this could be interpreted as increased heterotrophic activity with increased temperature. The observed variation in percent transport resulted more from variation in the dissolved fraction than in the more stable small particulate fraction, however, and the dissolved fraction showed a significant inverse relationship with temperature (Fig. 7). Thus, percent transport was high at high temperature because of low dissolved ^{14}C , and not because of high ^{14}C levels in small particles. In fact, small particulate ^{14}C varied little throughout the experiments considering the large variations in primary productivity and temperature (Fig. 7).

The biological interpretation of these relationships remains a problem for further research. While the linear regression of dissolved ^{14}C on temperature was significant for these data (Fig. 7), earlier data from Lake Bysjön (Coveney et al., 1977) showed no relationship (t-test of regression coefficient, $0.5 > p > 0.4$). Procedures used to obtain the two data sets were similar, but not identical, and the earlier experiments covered a wider range of temperature, chlorophyll a and bacterial numbers.

Addition of a mixture of small-molecular organic compounds (glycine, glucose and sodium glycolate) was tested as a way to prevent heterotrophic uptake of labeled products during *in situ* incubation. While the organic addition decreased small particulate ^{14}C in all cases, the increases in dissolved organic ^{14}C which occurred were not proportional, and in two cases dissolved ^{14}C was unchanged or even decreased (Fig. 5). Total ^{14}C uptake was significantly stimulated by organic addi-

tion on one occasion. These various effects demonstrate that organic addition was not successful in preventing heterotrophic utilization of released material. It can be concluded, however, that compounds other than those added were responsible for the major transport of ^{14}C -labeled material to small particles, since these high additions would have completely masked uptake of the same compound. Nalewajko & Lean (1972) looked at the size distribution (Sephadex) of extracellular products in natural water samples with and without addition of glycolate, glucose or acetate. They also found effects of organic addition on total ^{14}C uptake which complicated interpretation of the experiments, but they concluded that in some instances, addition of organic material allowed small molecular species to be detected which presumably were assimilated in the control sample.

Sharp (1977) questioned the practice of storing samples in darkness between incubation and filtration, as was done in the *in situ* experiments. Saunders (1972) investigated changes in extracellular organic ^{14}C in natural samples during the night following light incubation with H^{14}CO_3 . He showed that dissolved ^{14}C can continue to increase, remain constant or decrease during the dark period. Watt (1966) found increasing and erratic dissolved ^{14}C levels when natural samples were darkened after light H^{14}CO_3 incubation, but these experiments were accompanied by extremely high levels of dark release even in samples not first exposed to the light.

I tested the effects of dark storage during laboratory experiments by transferring a portion of the light sample to

a dark bottle after 3 h incubation. Release (dissolved + small particulate) continued ca. 1 h at a rate similar to that in the light, but decreased thereafter (data not shown). Total released ^{14}C increased about 25% during 2 h dark storage following 3 h incubation, and the effect of 2 h storage after 4 h incubation (i.e. *in situ*) should have been less. In practice, dark storage of samples is usually necessary, since stopping H^{14}CO_3 uptake with fixatives can give large losses of particulate ^{14}C (Silver & Davoll, 1978). That dark storage did not unduely affect the *in situ* data is also seen in the good agreement between values for percent dissolved and small particulate ^{14}C from *in situ* (dark storage before filtration) and laboratory (no storage) experiments (Fig. 3B). The question of dark excretion behavior after light incubation is important when attempts are made to estimate cycling of photosynthetic carbon through dissolved and small particulate pools on a 24 h basis, however.

Time-Series Incubations

In time-series experiments similar to those described here, Smith & Higgins (1978), Wiebe & Smith (1977a) and Larsson & Hagström (1979) found accumulation of dissolved ^{14}C to a constant value (after 1.5 to 7.0 h). This was interpreted as an increase in specific activity ($^{14}\text{C}/^{12}\text{C}$) of a steady state pool of dissolved algal products to isotopic equilibrium with inorganic carbon in the medium.

Of the three time-series experiments discussed here, only

that conducted 12 October showed a steady state pattern during a 5.5 h incubation period (Fig. 4). If the extracellular product pool is interpreted as a steady state system, the plateau value for dissolved released carbon actually indicates the size of the product pool (on 12 October $3.0 \text{ mgC} \cdot \text{m}^{-3}$), held at a constant level by balanced release and heterotrophic uptake (Wiebe & Smith, 1977a). The initial increase in dissolved ^{14}C is a measure of replacement of unlabeled algal products by labeled. This disappearance of unlabeled products can be fitted to an exponential expression $X_t = X_0 e^{-kt}$, where X_t and X_0 are the concentrations of unlabeled products at times t and 0. The inverse of the rate constant k is turnover time, which together with pool size gives the true release and uptake rate. For time-series experiment 12 October, turnover time was 1.2 h, and the release and uptake rate was $2.5 \text{ mgC} \cdot \text{m}^{-3} \cdot \text{h}^{-1}$ or 0.78% of total C uptake.

With the exception of a deviating value at 20 min, percent release values calculated as dissolved + small particulate ^{14}C during the experiment, 0.60 to 0.80%, were similar to that derived above from steady state kinetics, 0.78% (Fig. 4, 12 October). Thus two independent methods of estimating gross extracellular release gave equivalent values, which supports the interpretation of small particulate ^{14}C as microheterotrophic uptake of labeled algal products.

In the remaining two time-series experiments (14 September, 27 September), increase in dissolved ^{14}C was approximately linear over 5.5 h (Fig. 4). If the specific activity of the internal pools of material for release rapidly achieved

equilibrium, this essentially linear increase in extracellular ^{14}C with time indicates either 1) release at a rate faster than uptake - i.e. the pool of extracellular products was not at steady state, but increasing; or 2) release to a pool so large that its specific activity was still increasing linearly at 5.5 h. The fact that significant small particulate uptake was observed in samples at 20 min makes the first explanation, increasing pool size, the most likely, since the large initial pool necessary for the second mechanism would have greatly diluted the early labeled products.

Smith & Higgins (1978) found linear release over ca. 5 h in a non-axenic culture of *Isochrysis galbani* treated with antibiotics, while the untreated culture showed a plateau after ca. 2 h. This demonstrated that linear net release can occur when uptake is low. Nalewajko, Dunstall & Shear (1976) found linear extracellular release in axenic cultures of *Chlorella pyrenoidosa* and *Anabaena flos-aquae*. Infection of the cultures with different types and levels of bacteria decreased dissolved ^{14}C values compared to axenic controls, but plateau-type curves did not necessarily result. In two of four cases, dissolved ^{14}C still increased more-or-less linearly with time. Saunders (1972) measured H^{14}CO_3 uptake and net extracellular release *in situ* over 24 h light cycles for a variety of freshwater systems. His experiments were done under natural (varying) light, but in no case was there indication of a plateau in extracellular organic ^{14}C . The time-series experiments made by Lancelot (1979) showed both linear and plateau-type curves for dissolved ^{14}C release in

marine phytoplankton.

It appears from the time-series experiments done here and the literature cited that steady state conditions for algal extracellular product pools are not universal in natural plankton communities. Evidence for an increasing (non-steady state) extracellular pool is also seen in values for percent transport of released material to small particles (Fig. 3). For the *in situ* experiments, the same water sample was incubated at all depths. Given the lower absolute excretion rate in the deeper samples, isotopic equilibrium in a steady state extracellular pool would have been achieved slower than in surface samples. This would have resulted in a lower apparent percent transport with depth, but in fact transport values tended to remain constant or increase with depth. This was even true for *in situ* experiment 11 October, although steady state kinetics were seen in the laboratory on the following day. Algal biomass was ca. 45% higher in the water taken on 12 October for laboratory incubation than in that used the previous day in the field, due to a concentration of motile forms towards the lake surface (Table 1). There is no indication that this difference in biomass was related to the apparent difference in release and/or uptake behavior, however.

All results cited here have been based on the assumption that the specific activity of material for release rapidly reaches isotopic equilibrium. If the specific activity of the internal pool is increasing, dissolved ^{14}C may not reach a plateau within several hours, even though the extracellular pool is at steady state. Wiebe & Smith (1977a) rejected this

possibility for their data, and there is no indication of this in the present work. Unless there is information on the internal turnover time of material for excretion, however, the possibility cannot be excluded.

Storch & Saunders (1978) calculated high and low estimates of phytoplankton excretion assuming 1) release from a pool slow to achieve equilibrium (i.e. the intracellular protein pool), and 2) release from a pool at instantaneous equilibrium. The non-equilibrium model resulted in excretion rate estimates 2 to 7 times higher than those obtained when immediate equilibrium was assumed, which illustrates the size of the potential error. S ndergaard (1980) measured excretion of organic ^{14}C from macrophytes in flow-through chambers, which allowed him to follow changes in the specific activity of the products. Of the six species studied, one reached a constant ^{14}C release rate within 4 h, two required ca. 20 h, and three still showed an increasing rate of ^{14}C release after 24 h incubation. These differences were apparently related to the molecular size distribution of the products, where larger molecules required longer time to reach isotopic equilibrium. While carbon turnover is more rapid in phytoplankton than in macrophytes, it is probable that isotopic equilibrium for at least some extracellular products is not achieved during short-term labeling. Considering the large spectrum of products known to be released by algae (Fogg, 1971; Hellebust, 1974), the kinetics of ^{14}C release during incubation could become quite complex.

Microheterotrophic Uptake of Algal Extracellular Products

The ability of bacteria to utilize dissolved organic material in algal batch cultures is well documented (Nalewajko, 1977; Herbst & Overbeck, 1978). Mayfield & Inniss (1978) found that *Ankistrodesmus braunii* and a *Flavobacterium* strain could be grown together at steady state in continuous culture. Algal products were the sole source of carbon, and excretion rather than lytic products was implicated since algal growth rate was constant and the bacteria did not colonize algal cells.

Excretion and uptake of algal products has also been demonstrated directly in natural planktonic communities. One approach is to prepare labeled excretion products by incubation of the natural community with H^{14}CO_3 . The products are then incubated with the original water sample to measure uptake. Any compound subject to rapid bacterial utilization will be depleted in the labeled product mixture, however, which will result in underestimation of subsequent uptake. Storch & Saunders (1978), for example, measured uptake in a eutrophic lake of algal products prepared using 24 h incubation. Uptake followed first order kinetics but was at most ca. $2\% \text{ h}^{-1}$ (calculated from Fig. 14; Storch, 1971). Using a similar procedure, but with a 6 h incubation to prepare labeled products, Iturriaga & Hoppe (1977) measured uptake of about 8, 12 and $18\% \text{ h}^{-1}$ for three samples from the Baltic Sea. In a further development of this procedure, Wiebe & Smith (1977a) size fractionated and concentrated the phytoplankton

to prepare labeled products at high specific activity and, presumably, with low loss to bacteria. Since the extracellular pool was found to be at steady state in their experiments, uptake was 100% of release. A problem with their tracer method is the risk that the many manipulations of the phytoplankton required for tracer preparation might give a labeled product mixture which is not representative for natural conditions (see discussion in Wiebe & Smith, 1977b).

Unless the kinetics of release and uptake are known in detail, measurements with labeled product tracers cannot be compared directly with results from size fractionation experiments, which give an indirect estimate of the uptake of algal excretion products occurring simultaneously with release. Derenbach & Williams (1974) used size fractionation to estimate bacterial uptake of algal extracellular products in the English Channel. Using a 3 μm membrane filter for fractionation, they found ^{14}C in the small particulate fraction to be 1 to 23% of total particulate ^{14}C after 4 to 6 h incubations. Larsson & Hagström (1979) used fractionation with 3 μm Nuclepore filters to estimate bacterial uptake of algal products in the Baltic Sea. They found an annual mean of 27% of ^{14}C uptake per unit area in the small particulate fraction after 4 h *in situ* incubation with H^{14}CO_3 . These values are considerably higher than the 0.75 to 2.6% of ^{14}C uptake per unit area found in the small particulate fraction after 4 h incubation in Lakes Bysjön and Häljasjön (Fig. 3).

Contamination of the small particulate size fraction with algal cells would give high apparent bacterial ^{14}C . The com-

mon occurrence of very small algal forms and the passage of a large percentage of the chlorophyll *a* in natural water samples through 3 μ m Nuclepore filters have been shown both for marine (Berman, 1975; Azam & Hodson, 1977; Johnson & Sieburth, 1979) and fresh (Paerl, 1977; Berman & Stiller, 1977; Riemann, 1978) waters. Thus the routine use of a 3 μ m filter to separate autotrophic and heterotrophic organisms is questionable. While Derenbach & Williams (1974) did not monitor the small particulate fraction of their samples for algal cells, Larsson & Hagström (1979) found no algal cells when the 3 μ m filtrate was examined by scanning electron microscopy.

The empirical observation has been made that percent dissolved ^{14}C (net excretion) is higher in oligotrophic systems than in more eutrophic waters (Watt, 1966; Berman & Holm-Hansen, 1974), although the data are by no means unambiguous (Williams & Yentsch, 1976). While no mechanism has yet been shown to explain these general differences, the low net excretion in the eutrophic lakes considered here is certainly consistent. Percent assimilated carbon in the bacterial fraction after ca. 4 h incubation might be higher in the systems studied by Derenbach & Williams (1974) and Larsson & Hagström (1979) because percent extracellular release is higher.

Calculation of bacterial production from algal products using size fractionation data requires several assumptions, the more important of which concern the following questions:

- 1) How fast does the intracellular pool of material to be released reach isotopic equilibrium?
- 2) How fast does the extracellular product pool reach isotopic equilibrium?
- 3) Does all accumulation of ^{14}C in small particles represent bacterial uptake of algal products?
- 4) How much of the released carbon taken up by bacteria is respired?
- 5) What is the excretion behavior of the phytoplankton over a 24 h light/dark cycle?

Derenbach & Williams (1974) concluded that gross bacterial production amounted to from 20 to over 50% of ^{14}C primary production, although they assumed continued release and uptake of organic carbon in the dark. Larsson & Hagström (1979) estimated daytime bacterial production to 25% (net) and 45% (gross) of ^{14}C primary production on an annual basis. They assumed steady state conditions for the extracellular pool, however, which was not indicated by the present data.

The data from Lakes Bysjön and Häljasjön were not sufficient to answer all of the questions above. However, with the assumption that small particulate ^{14}C represents bacterial uptake of algal products, then this is a minimum estimate of net bacterial production from algal products during incubation. Correction of the small particulate ^{14}C for respiration losses or delay in attainment of isotopic equilibria would increase the uptake estimate. For comparison, dark uptake of $^{14}\text{CO}_2$ was used to estimate total heterotrophic bacterial production (Overbeck, 1979). Small particulate ^{14}C

in formalin-fixed blanks was subtracted from small particulate dark ^{14}C fixation, and heterotrophic bacterial production was estimated by assuming that 4% of carbon production is fixed as CO_2 . While this factor is variable, 3 to 5% is typical for aerobic heterotrophs using easily oxidized organic matter (Overbeck, 1979).

For the four autumn *in situ* experiments, uptake of algal carbon by microheterotrophs in surface water amounted to 32, 95, 56 and 56% of the heterotrophic production as estimated by dark $^{14}\text{CO}_2$ uptake (Table 5). This indicates that, at least for selected depths at midday, bacterial uptake of algal products can be a sizeable fraction of total bacterial production. These data are from productive lakes, where concentrations of total dissolved organic carbon are high (in Lake Bysjön typically 15 to 20 mgC l^{-1} during summer). Thus phytoplankton excretion, while apparently not an important carbon loss for the algae, may represent an important carbon source for the planktonic bacteria if it is a qualitatively more suitable substrate than the background dissolved organic matter.

The laboratory and field experiments in Lake Bysjön indicated non-steady state conditions for the extracellular pool during algal photosynthesis. However, these products most certainly do not accumulate over longer time periods. If production exceeds uptake in the light, then the situation should be reversed in the dark, and pool size would fluctuate on a diel basis. Saunders & Storch (1971) postu-

lated this kind of diel pattern for extracellular products, and supported their hypothesis experimentally using glucose to simulate algal excretion. Further knowledge of diel cycles *in situ* is necessary, since extrapolation of short-term measurements of release and uptake to 24 h periods is central to an understanding of these processes in natural systems.

ACKNOWLEDGEMENTS

I thank Prof. S. Björk; Dr. B. Riemann and Dr. A. Södergren for reading the manuscript and suggesting numerous improvements. I also thank Dr. G. Cronberg for identifying the phytoplankton and Prof. L.O. Björn for use of the liquid scintillation counter and spectrofluorometer. Portions of this work were supported by the Swedish Natural Science Research Council (NFR) and the National Swedish Environment Protection Board (SNV).

REFERENCES

- Anon. (1974) Soluene-350 Soluene-100 ... tissue solubilizers. Packard Instrument Co., Inc. 3004/0755-15. 8 pp.
- Azam F. & Hodson R.E. (1977) Size distribution and activity of marine microheterotrophs. *Limnology and Oceanography* 22, 492-501.
- Berman T. (1975) Size fractionation of natural aquatic populations associated with autotrophic and heterotrophic carbon uptake. *Marine Biology* 33, 215-220.
- Berman T. & Holm-Hansen O. (1974) Release of photoassimilated carbon as dissolved organic matter by marine phytoplankton. *Marine Biology* 28, 305-310.
- Berman T. & Stiller M. (1977) Simultaneous measurement of phosphorus and carbon uptake in Lake Kinneret by multiple isotopic labeling and differential filtration. *Microbial Ecology* 3, 279-288.
- Chróst R.J. (1978) The estimation of extracellular release by phytoplankton and heterotrophic activity of aquatic bacteria. *Acta Microbiologica Polonica* 27, 139-146.
- Coveney M.F. (1978) Separation of algae and bacteria in lake water by size fractionation. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 20, 1264-1269.
- Coveney M.F., Cronberg G., Enell M., Larsson K. & Olofsson L. (1977) Phytoplankton, zooplankton and bacteria - standing crop and production relationships in a eutrophic lake. *Oikos* 29, 5-21.

- Derenbach J.B. & Williams P.J. Le B. (1974) Autotrophic and bacterial production: Fractionation of plankton populations by differential filtration of samples from the English Channel. *Marine Biology* 25, 263-269.
- Dunstall T.G. & C. Nalewajko (1975) Extracellular release in planktonic bacteria. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 19, 2643-2649.
- Fogg G.E. (1971) Extracellular products of algae in fresh-water. *Archiv für Hydrobiologie. Beihefte. Ergebnisse der Limnologie* 5, 1-25.
- Goldman C.R., Steemann Nielsen E., Vollenweider R.A. & Wetzel R.G. (1974) The ^{14}C light and dark bottle technique. In: *A Manual on Methods for Measuring Primary Production in Aquatic Environments* (Ed. R.A. Vollenweider). *International Biological Program Handbook No. 12*, 2nd edition. Blackwell, Oxford. 225 pp.
- Hellebust J.A. (1974) Extracellular products. In: *Algal Physiology and Biochemistry* (Ed. W.D.P. Stewart). *Botanical Monographs* 10. Blackwell, Oxford. 989 pp.
- Herbst V. & Overbeck J. (1978) Metabolic coupling between the alga *Oscillatoria redekei* and accompanying bacteria. *Naturwissenschaften* 65, 598-599.
- Hobbie J.E., Daley R.J. & Jasper S. (1977) Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Applied and Environmental Microbiology* 33, 1225-1228.
- Hobbie J.E. & Wright R.T. (1979) An assessment of quantitative measurements of aquatic microbes. *Archiv für Hydrobiologie*.

- Beihefte. Ergebnisse der Limnologie 13, 85-95.
- Holm-Hansen O., Lorenzen C.J., Holmes R.W. & Strickland J.D.H. (1965) Fluorometric determination of chlorophyll. Journal du Conseil permanent international pour l'Exploration de la Mer 30, 3-15.
- Iturriaga R. & Hoppe H.-G. (1977) Observations of heterotrophic activity on photoassimilated organic matter. Marine Biology 40, 101-108.
- Johnson P.W. & Sieburth J.McN. (1979) Chroococcoid cyanobacteria in the sea: A ubiquitous and diverse phototrophic biomass. Limnology and Oceanography 24, 928-935.
- Lancelot C. (1979) Gross excretion rates of natural marine phytoplankton and heterotrophic uptake of excreted products in the southern North Sea, as determined by short-term kinetics. Marine Ecology - Progress Series 1, 179-186.
- Larsson U. & Hagström A. (1979) Phytoplankton exudate release as an energy source for the growth of pelagic bacteria. Marine Biology 52, 199-206.
- Lean D.R.S. & Burnison B.K. (1979) An evaluation of errors in the ^{14}C method of primary production measurement. Limnology and Oceanography 24, 917-928.
- Lorenzen C.J. (1967) Determination of chlorophyll and phaeopigments: spectrophotometric equations. Limnology and Oceanography 12, 343-346.
- Mayfield C.I. & Inniss W.E. (1978) Interactions between freshwater bacteria and *Ankistrodesmus braunii* in batch and continuous culture. Microbial Ecology 4, 331-344.
- Nalewajko C. (1977) Extracellular release in freshwater algae

- and bacteria: extracellular products of algae as a source of carbon for heterotrophs. In: Aquatic Microbial Communities (Ed. J. Cairns, Jr.). Garland Publishing, Inc., N.Y. 693 pp.
- Nalewajko C., Dunstall T.G. & Shear H. (1976) Kinetics of extracellular release in axenic algae and in mixed algal-bacterial cultures: significance in estimation of total (gross) phytoplankton excretion rates. *Journal of Phycology* 12, 1-5.
- Nalewajko C. & Lean D.R.S. (1972) Growth and excretion in planktonic algae and bacteria. *Journal of Phycology* 8, 361-366.
- Overbeck J. (1979) Dark CO₂ uptake - biochemical background and its relevance to *in situ* bacterial production. *Archiv für Hydrobiologie. Beihefte. Ergebnisse der Limnologie* 12, 38-47.
- Paerl H.W. (1977) Ultraphytoplankton biomass and production in some New Zealand lakes. *New Zealand Journal of Marine and Freshwater Research* 11, 297-305.
- Rebsdorf A. (1972) The carbon dioxide system in freshwater. Freshwater Biological Laboratory. Hillerød, Denmark.
- Riemann B. (1978) Differentiation between heterotrophic and photosynthetic plankton by size fractionation, glucose uptake, ATP and chlorophyll content. *Oikos* 31, 358-367.
- Saraceni C. & Ruggiu D. (1974) Techniques for sampling water and phytoplankton. In: A Manual on Methods for Measuring Primary Production in Aquatic Environments (Ed. R.A. Vollenweider). International Biological Program Handbook

No. 12, 2 nd edition. Blackwell, Oxford. 225 pp.

Saunders G.W., Jr. (1972) The kinetics of extracellular release of soluble organic matter by plankton. *Verhandlungen der Internationalen Vereinigung für theoretische und angewandte Limnologie* 18, 140-146.

Saunders G.W. & Storch T.A. (1971) Coupled oscillatory control mechanism in a planktonic system. *Nature* 230, 58-60.

Sharp J.H. (1977) Excretion of organic matter by marine phytoplankton: Do healthy cells do it? *Limnology and Oceanography* 22, 381-399.

Silver M.W. & Davoll P.J. (1978) Loss of ^{14}C activity after chemical fixation of phytoplankton: Error source for autoradiography and other productivity measurements. *Limnology and Oceanography* 23, 362-368.

Smith D.F. & Higgins H.W. (1978) An interspecies regulatory control of dissolved organic carbon production by phytoplankton and incorporation by microheterotrophs. In: *Proceedings in Life Sciences, Microbial Ecology, First International Symposium 1977* (Ed. M.W. Loutit & J.A.R. Miles). Springer-verlag, Berlin.

Smith D.F. & Wiebe W.J. (1976) Constant release of photosynthate from marine phytoplankton. *Applied and Environmental Microbiology* 32, 75-79.

Søndergaard M. (1980) Carbon Metabolism in submerged freshwater macrophytes. University of Aarhus, Aarhus, Denmark. Ph.D. Dissertation. 148 pp.

Steemann Nielsen E. (1952) The use of radioactive carbon (C-14) for measuring organic production in the sea. *Journal*

du Conseil permanent international pour l'Exploration de la Mer 18, 117-140.

Storch T.A. (1971) Production of extracellular dissolved organic carbon by phytoplankton and its utilization by bacteria. University of Michigan, Ann Arbor, Michigan, USA. Ph.D. Dissertation. 147 pp.

Storch T.A. & Saunders G.W. (1978) Phytoplankton extracellular release and its relation to the seasonal cycle of dissolved organic carbon in a eutrophic lake. Limnology and oceanography 23, 112-119.

Watt W.D (1966) Release of dissolved organic material from the cells of phytoplankton populations. Proceedings of the Royal Society. London. Series B 164, 521-551.

Wiebe W.J. & Smith D.F. (1977a) Direct measurement of dissolved organic carbon release by phytoplankton and incorporation by microheterotrophs. Marine Biology 42, 213-223.

Wiebe W.J. & Smith D.F. (1977b) ^{14}C -labeling of the compounds excreted by phytoplankton for employment as a realistic tracer in secondary productivity measurements. Microbial Ecology 4, 1-8.

Williams P.J.Le B. & Yentsch C.S. (1976) An examination of photosynthetic production, excretion of photosynthetic products, and heterotrophic utilization of dissolved organic compounds with reference to results from a coastal subtropical sea. Marine Biology 35, 31-40.

Table 1. Background data for the *in situ* and laboratory experiments.

				Integrated water sample 0 to 2 m depth		
Experiment	Type	Lake	Temp. (C)	Chl <i>a</i> (mg·m ⁻³)	Bacteria (10 ⁹ cells·l ⁻¹)	Dominant algal taxa ¹
29 March	<i>in situ</i>	L. Bysjön	5.4	60.5	11	Stephanodiscus hantzschii Grun. Cryptomonas sp.
3 April	<i>in situ</i>	L. Bysjön	6.2	35.4	10	Cryptomonas sp. Stephanodiscus hantzschii
12 April	<i>in situ</i>	L. Bysjön	7.6	31.1	6.1	Cryptomonas sp. undetermined nanoalgae
7 Sept.	<i>in situ</i>	L. Bysjön	15.8	28.5	9.6	Ceratium hirundinella (O.F. Müller) Schrank Aphanizomenon flos-aquae (L.) Ralfs Melosira granulata (Ehrenb.) Ralfs var. angust. Müller
13 Sept.	<i>in situ</i>	L. Bysjön	15.2	60.4	8.5	(as 7 Sept.)
14 Sept	lab.	L. Bysjön	-	60.7	11	(as 7 Sept.)
26 Sept.	<i>in situ</i>	L. Häljasjön	12.9	27.6	9.4	Aphanizomenon flos-aquae Melosira spp. Cryptomonas sp.
27 Sept.	lab.	L. Häljasjön	-	30.8	8.5	(as 26 Sept.)
11 Oct.	<i>in situ</i>	L. Bysjön	11.8	55.1	12	Pandorina morum (Müller) Bory Melosira granulata v. angust. Ceratium hirundinella
12 Oct.	lab.	L. Bysjön	-	80.4	12	(as 11 Oct.)

1. In order of importance based on estimated biovolume.

Table 2. Algal pigments and carbon ($H^{14}CO_3$) uptake in small particulate fraction as percent of that in whole water sample for field incubations. Pigments measured in acetone extracts both as total fluorescence and as chl a . Incubation for carbon uptake at 6.5 m depth with fractionation before (pre-) and after (post-) incubation. Both light and light minus dark values shown for carbon uptake.

Experiment	Content in small particulate fraction as % of whole water content					
	Algal pigment		Carbon uptake, pre-fract.		Carbon uptake, post-fract.	
	Total fluor. (%)	Chl a (%)	Light (%)	Light-dark (%)	Light (%)	Light-Dark (%)
29 March	0.10	0.042	-	-	0.91	0.85
3 April	0.16	0.059	-	-	1.9	1.8
12 April	0.23	0.11	-	-	1.4	1.4
7 Sept.	0.98	0.77	0.55	0.061	2.4	2.1
13 Sept.	0.20	0.14	0.12	0.019	1.0	0.94
26 Sept.	1.2	0.86	0.15	0.00	1.1	1.0
11 Oct.	0.27	0.092	0.15	0.053	0.52	0.46

- : not analyzed

Table 3. Percent bacteria passing the fractionation filter (Whatman GF/C). On three occasions, samples were also fractionated after dark incubation with ^{14}C -glucose.

<u>Experiment</u>	<u>Percent bacteria in GF/C filtrate (%)</u>	<u>Percent ^{14}C-glucose uptake activity in GF/C filtrate (%)</u>
29 March	80	-
3 April	70	-
12 April	84	-
7 Sept.	62	-
13 Sept.	77	-
14 Sept.	66	65
26 Sept.	70	-
27 Sept.	62	43
11 Oct.	83	-
12 Oct.	81	61

- : not analyzed

Table 4. ^{14}C retained on a 0.2 μm filter during refiltration of a 0.2 μm filtrate through the GF/C + 0.2 μm filter combination used for fractionation. ^{14}C retained upon refiltration is expressed as:

- A) Percent of total organic ^{14}C in the first filtrate.
 B) Percent of ^{14}C retained during the first filtration on the 0.2 μm filter of a GF/C + 0.2 μm filter combination.

Mean values for A and B are 6.6% and 5.6%, respectively (7 Sept., 0.5 m excluded).

<u>Experiment</u>	<u>Exposure depth (m)</u>	<u>^{14}C retained during refiltration</u>	
		<u>A (%)</u>	<u>B (%)</u>
7 Sept.	0.5	32	19
	0.1	9.3	4.6
13 Sept.	0.5	7.9	4.7
	0.1	7.4	3.8
26 Sept.	0.5	5.9	9.3
	0.1	4.7	5.3
11 Oct.	0.5	7.3	6.4
	0.1	3.6	4.9

Table 5. Microheterotrophic uptake of labeled algal products *in situ* compared to total bacterial production as estimated by dark $^{14}\text{CO}_2$ uptake in the small particulate fraction. Uptake of labeled products was taken as the maximum measured in each vertical profile.

Experiment	Dark uptake ($\text{mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$)	Bacterial production ($\text{mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$)	Small particulate uptake	
			($\text{mgC}\cdot\text{m}^{-3}\cdot\text{h}^{-1}$)	Percent of production (%)
7 Sept.	1.03×10^{-1}	2.57	8.10×10^{-1}	32
13 Sept.	4.97×10^{-2}	1.24	1.18	95
26 Sept.	5.34×10^{-2}	1.34	7.51×10^{-1}	56
11 Oct.	6.02×10^{-2}	1.51	8.47×10^{-1}	56

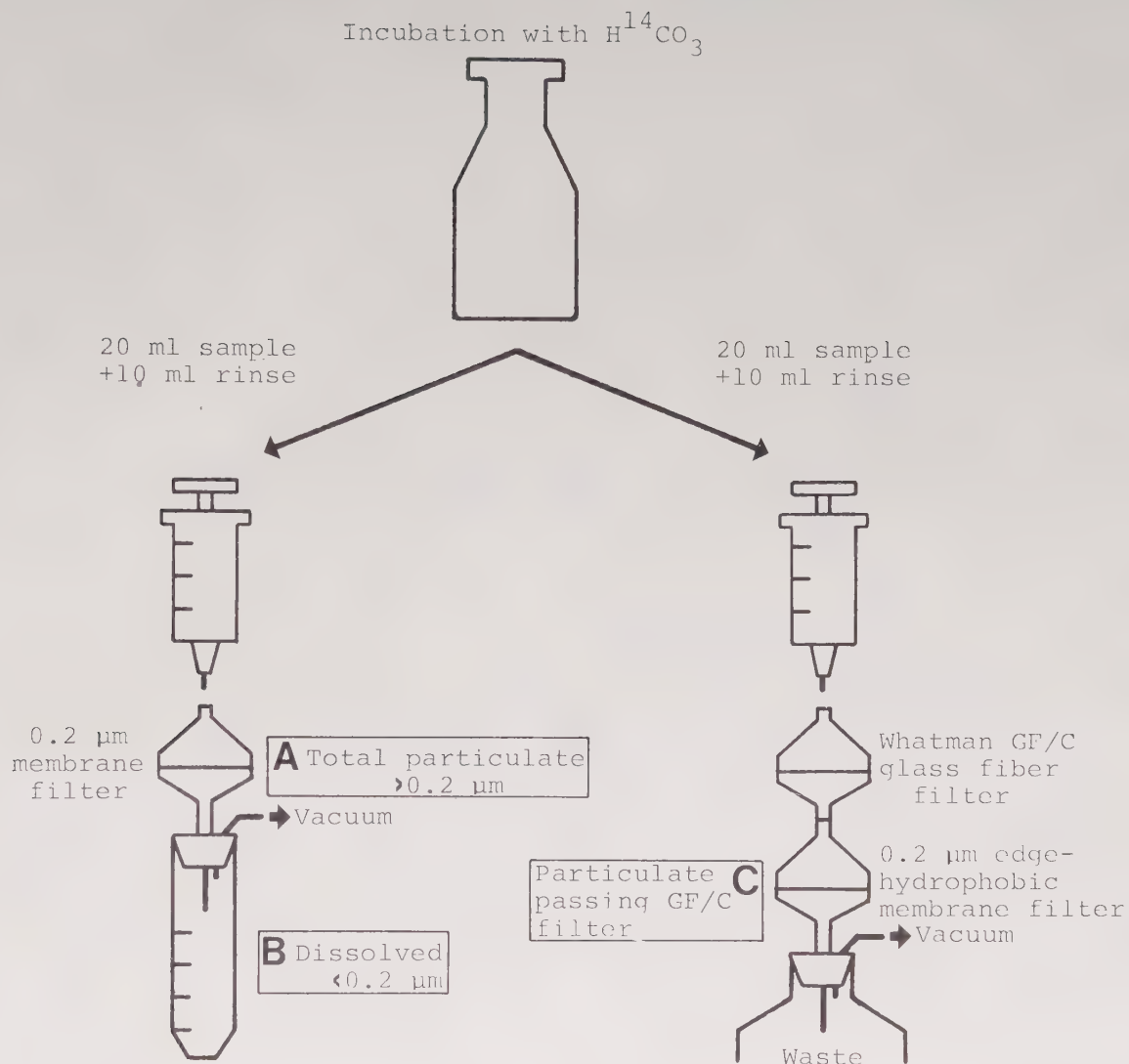


Figure 1. Schematic diagram of routine filtration procedures. ^{14}C activity was determined on membrane filters A and C and in filtrate B. All filters were in Millipore Swinnex holders. The edge-hydrophobic filter was used in the double holder to prevent air-lock.

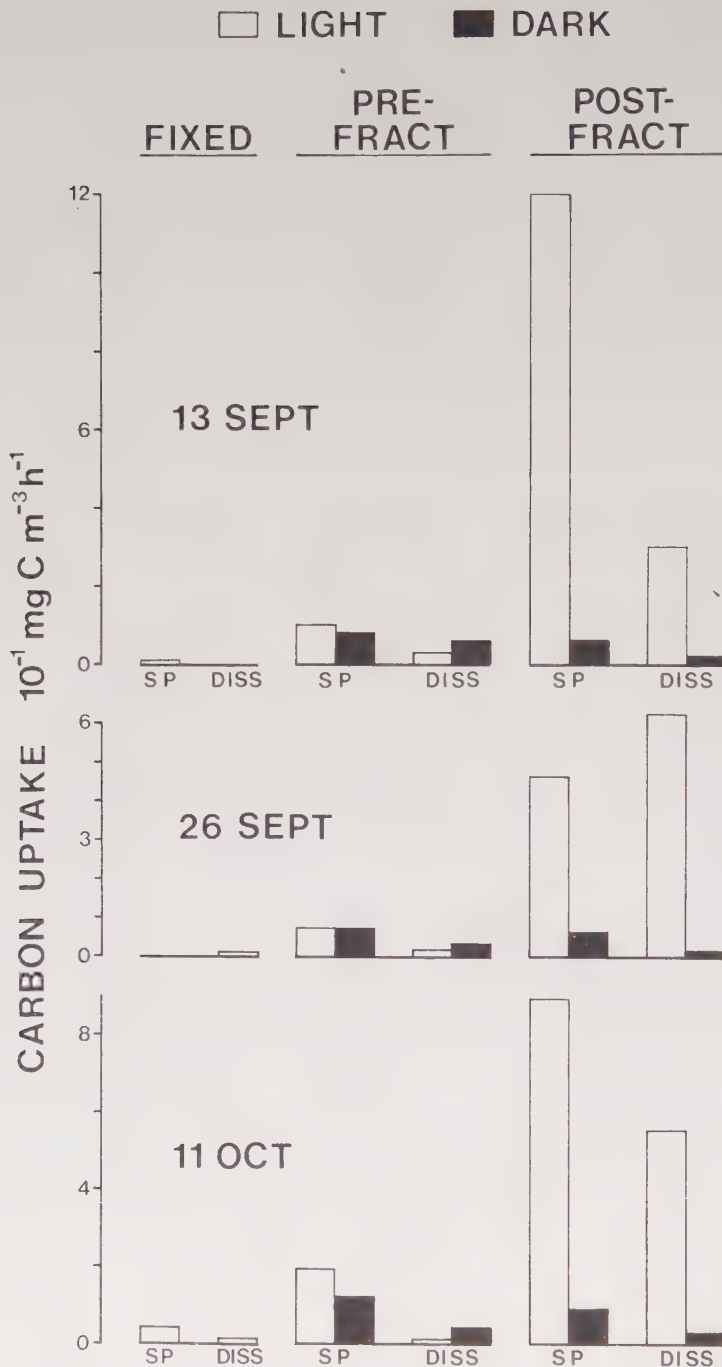


Figure 2. Carbon fixation into small particulate (SP) and dissolved (DISS) fractions during *in situ* incubation with H^{14}CO_3 . Samples fractionated before incubation (pre-fractionated) and after incubation (post-fractionated) were compared with formalin-fixed blanks (post-fractionated). Fixed samples were incubated in light only, while both light and dark incubations were done for the other treatments. Missing bars indicate values too small to plot.

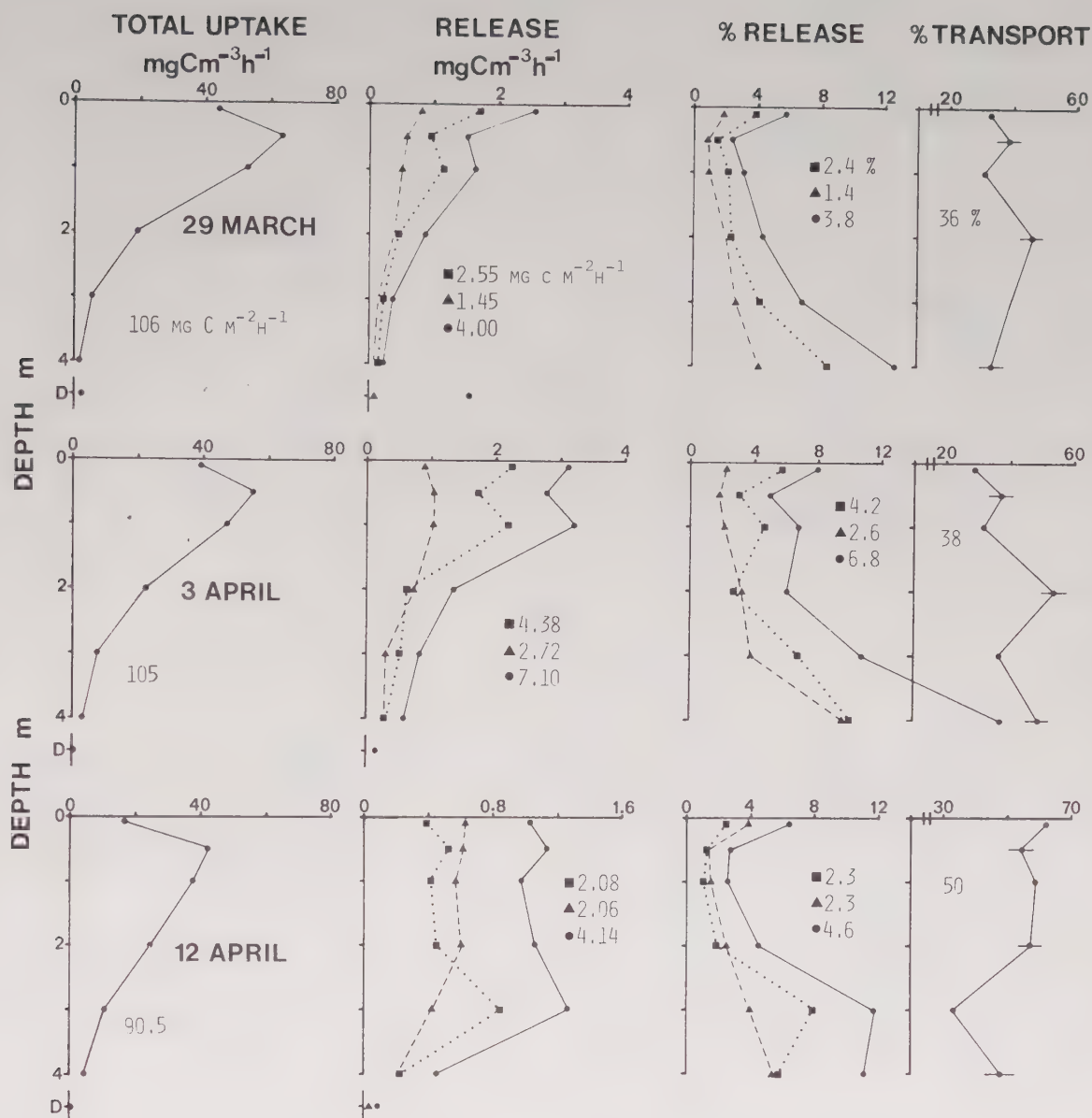


Figure 3A. Carbon (H^{14}CO_3) uptake in 4 h *in situ* incubations during spring 1978. Released organic carbon was recovered in dissolved (■····■) or small particulate (▲—▲) fractions, where (●—●) represents the sum of the two. D and B (Fig. 3B) on depth axes indicate dark bottles and formalin-fixed blanks, respectively. Horizontal bars under percent transport of released carbon to small particles show 95% CL. Uptake per m^2 lake surface is shown beside each profile. Some points for dark and blank samples are omitted for lack of space.

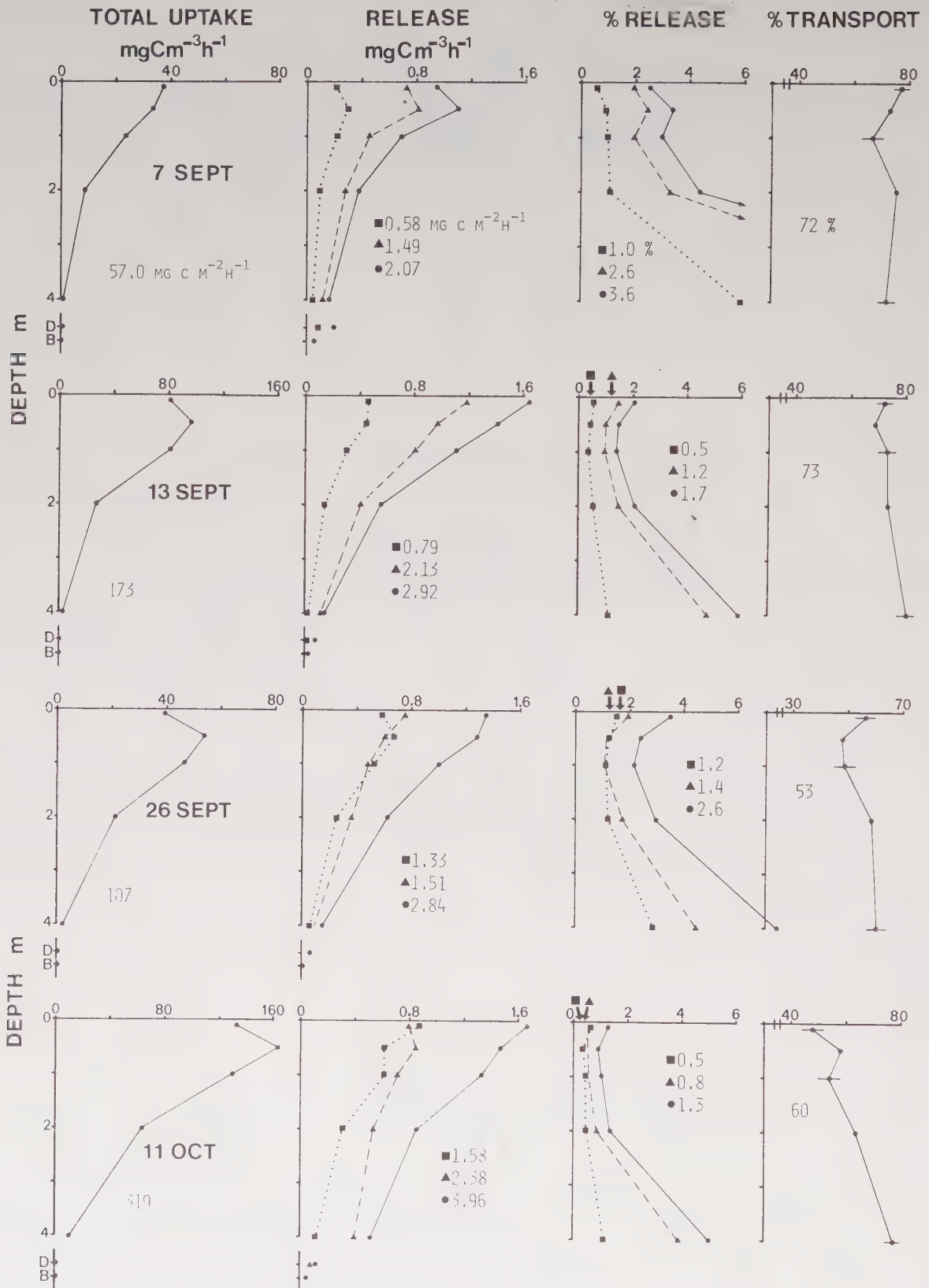


Figure 3B. Carbon (H^{14}CO_3) uptake in 4 h *in situ* incubations during fall 1978. See Fig. 3A for explanation of symbols. Arrows in final 3 experiments indicate percent dissolved and small particulate ^{14}C obtained in laboratory incubations at a light intensity equivalent to 0.8, 0.9 and 1.4 m for 13 Sept., 26 Sept. and 11 Oct., respectively.

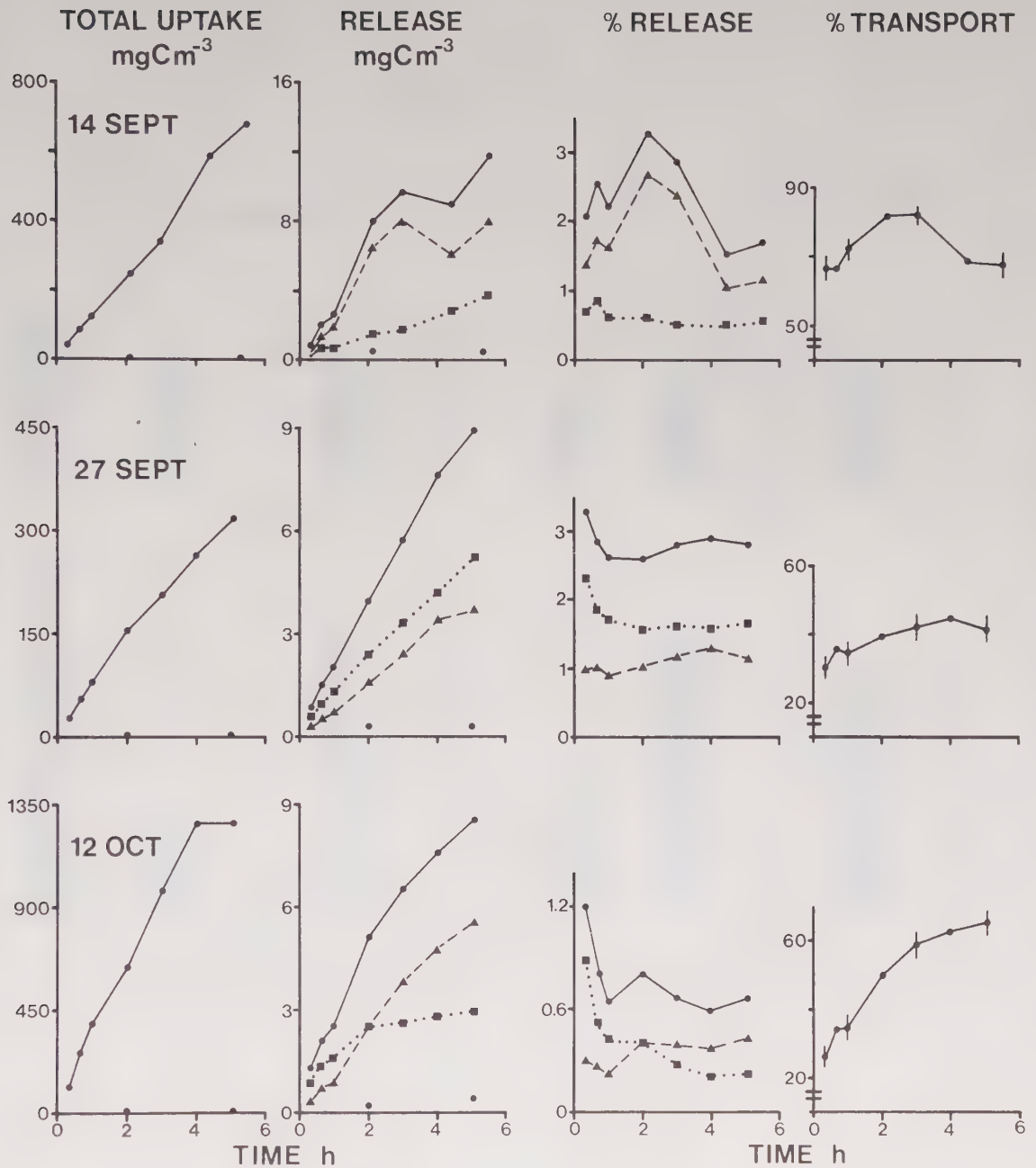


Figure 4. Carbon ($H^{14}CO_3$) uptake in time-series incubations. Released organic carbon was recovered in dissolved (■...■) or small particulate (▲—▲) fractions, where (●—●) represents the sum of the two. Vertical bars under percent transport of released carbon to small particles show 95% CL. Extra points at ca. 2 and 5 h show dark values for total uptake and release.

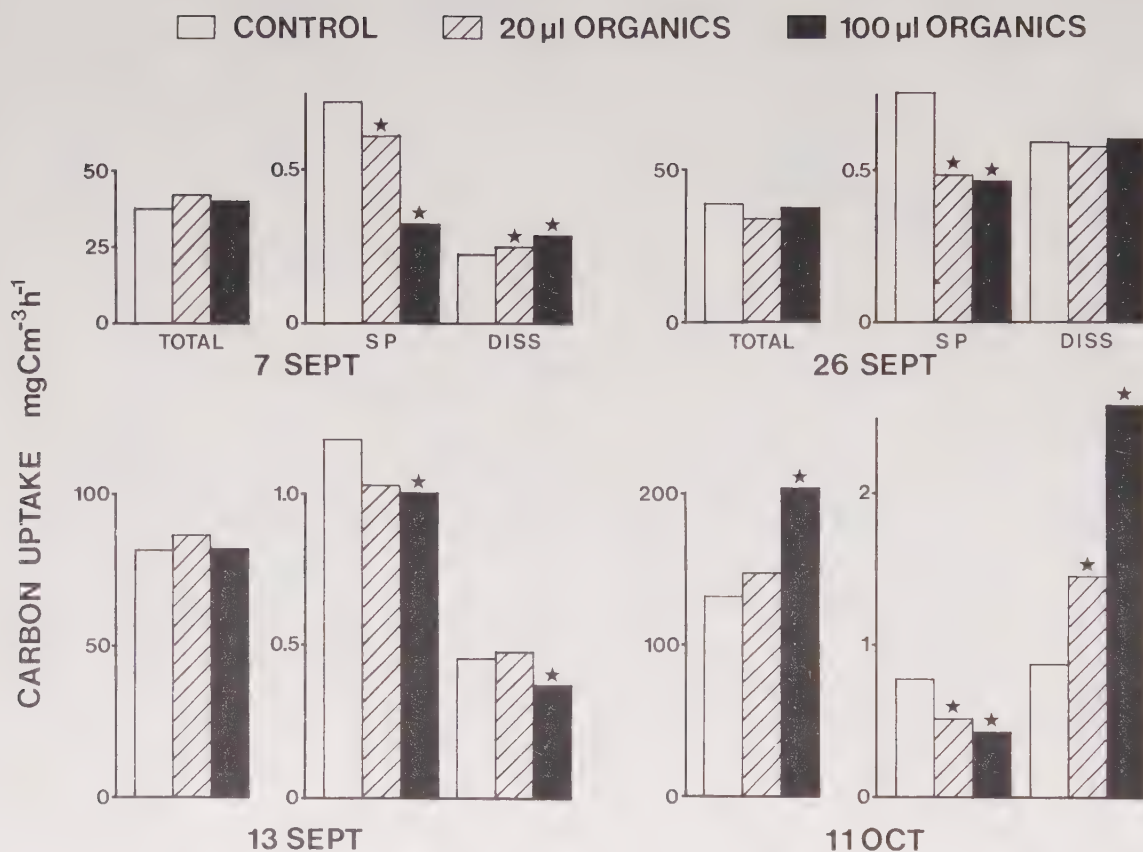


Figure 5. Carbon (H^{14}CO_3) uptake in size fractions of untreated controls and of samples receiving two levels of an organic mixture. Total assimilation (TOTAL) and the small particulate (SP) and dissolved (DISS) fractions are shown. Treatment values significantly different from the control (t-test, $p < 0.05$) are indicated (*).

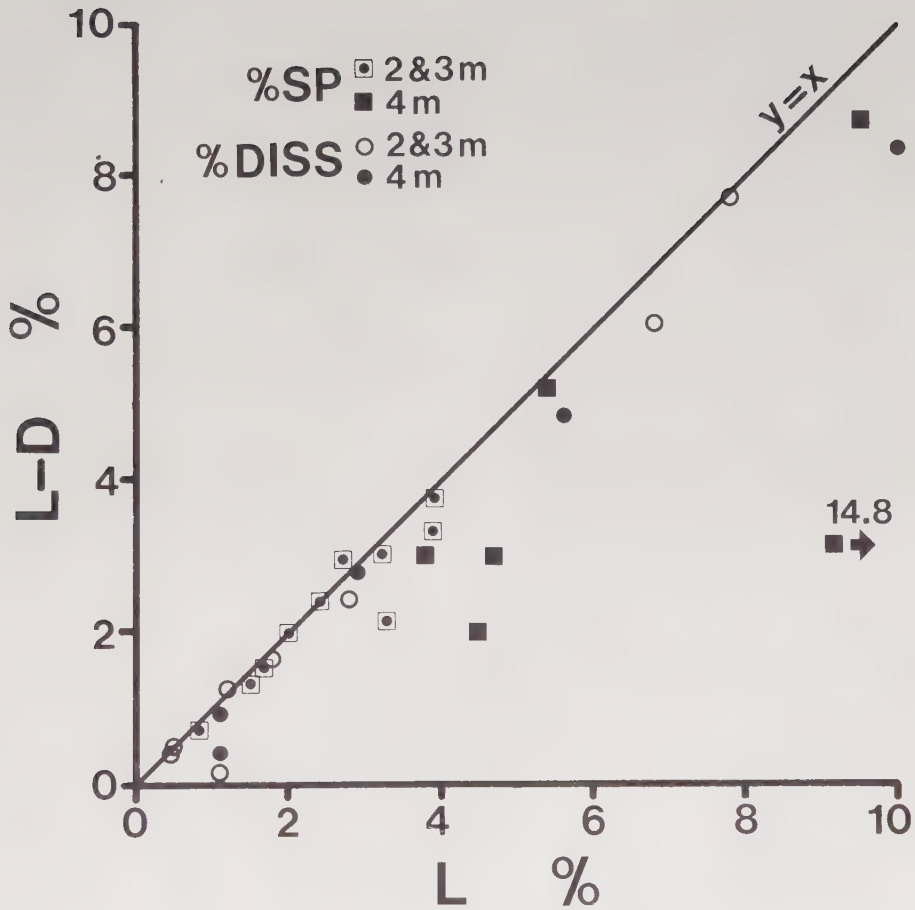


Figure 6. Percent small particulate (%SP) and percent dissolved (%DISS) ^{14}C calculated from light values (L) and light minus dark values (L-D) for light-limited depths of *in situ* experiments (2, 3 and 4 m). Line shows 1:1 relationship. $n = 29$.

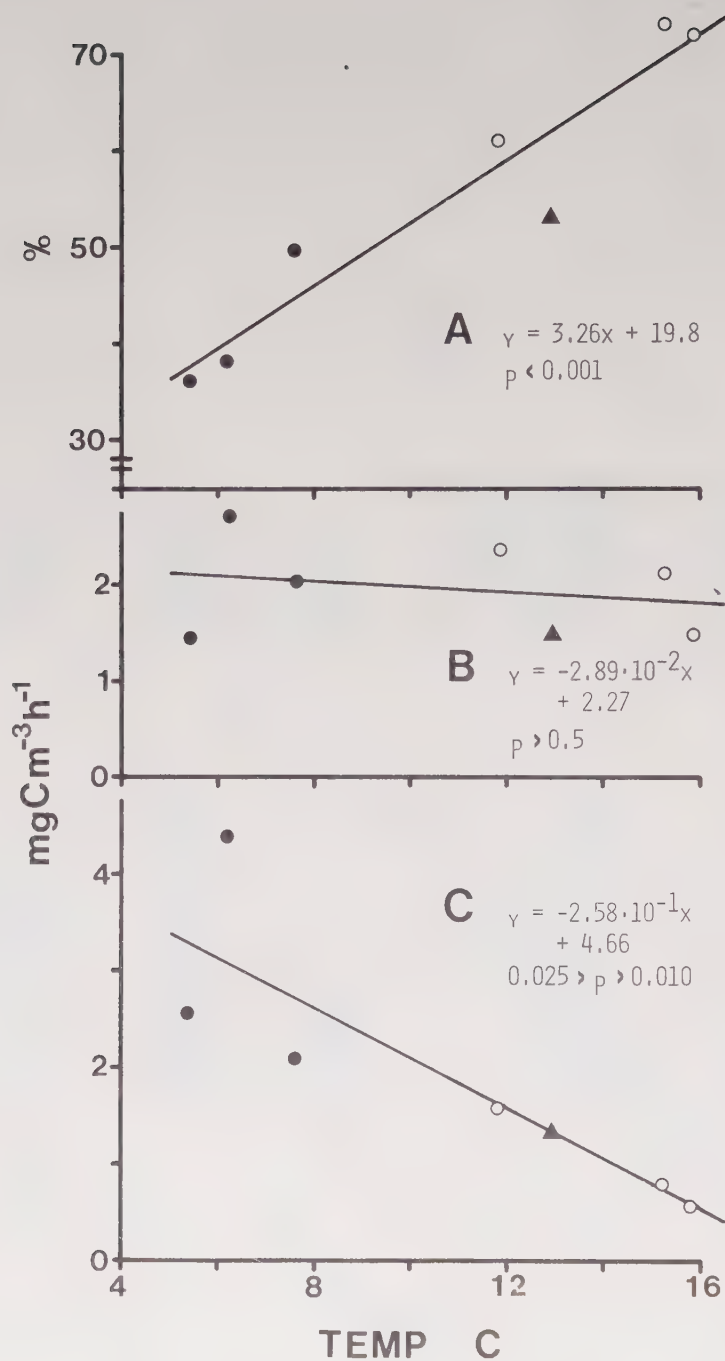


Figure 7. Linear regression of (A) percent transport and of carbon uptake to (B) small particulate and (C) dissolved fractions on *in situ* temperature. Values are per m^2 lake surface. (●) L. Bysjön, spring series; (○) L. Bysjön, fall series; (▲) L. Häljasjön. Significance levels (t-test) are shown for regression coefficients.

Handwritten text at the top of the page, possibly a title or header.

